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## RANDOMNESS AND DIRECTIVENESS IN THE EVOLUTION AND ACTIVITY OF LIVING ORGANISMS

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IN considering the problem of the origin and evolution of living organisms it is important to take into account the observed general characters of non-living events and systems as well as those peculiar to living organisms themselves, apparently the most highly evolved emergent systems in physical nature. Since Darwin, biological evolution has been regarded as based on the variability of individuals and the survival and perpetuation of favorable variations. We must remember, however, that this variability of living species has its parallel in non-living systems; the individuals of any inorganic class are found on close examination to be not exactly alike, even under conditions purposely made as uniform as possible. This is why the constants of natural science are defined not in terms of single observations but of averages based on large numbers. The scientific characterization is a statement of what will *probably* be found (approximately) when a given individual is examined or measured. The non-living as well as the living class-unit is to be regarded as always deviating, even if slightly, from the type as scientifically defined; each unit has its own "individual-

ity." Concrete individuality is always more or less at variance with constancy; any theoretical construct in natural science is an approximation and a compromise—a partial, not a complete, representation of the actual reality. The formula, scheme or model may represent the natural fact more or less adequately in some of its aspects,<sup>1</sup> but not in its full reality.

Usually the individual variations are regarded as examples of random deviation from a stable condition or "norm." By "random" we mean that they are as likely to occur in one direction as in another; in the long run they are distributed symmetrically about a mean, as illustrated in the tossing of coins, shaking of dice, shuffling, mixing and similar operations. The point especially to be noted is that the relative frequencies of these chance-determined variations in events of a given type show characteristically a high degree of constancy when large numbers are taken; accordingly, these frequencies are representable by a standard curve or formula. The science of probability is based upon this empirical constancy; it is a systematic formulation of what is observed to happen with sufficient instances; for example, the frequencies of the six possible dice-throws are experimentally found equal in good dice. But each single throw has its individuality; it is a unique event, and its precise outcome (to say nothing of the detail determining that outcome) cannot be predicted. In spite of this individual uncertainty, the stability of probability conditions is so great that they are now regarded as underlying the stability of many fundamental physical constants, especially those statistically based, as illustrated by the thermal and gas laws, quantum laws, radioactive properties of elements, and so on. In contrast to this symmetrical distribution of chance-determined events, directed events occur predominantly in some preferred direction or directions. Since

<sup>1</sup> Not all aspects, as we can discover by following up our examination in closer detail; eventually this discloses features characteristic of the individual but not of the class. Here scientific observation reaches its limit.

directiveness is the most characteristic feature of living organisms,<sup>1</sup> as shown in both their organization and their activity, the determination of its special factors is evidently a matter of fundamental importance to biology.

Uniformity based on the symmetrical distribution of undirected events in space and time is found everywhere in the natural world. In all material systems there is present the continual undercurrent of thermal activity, referred to the vibratory motions and inertia of molecules and atoms. Thermodynamic theory regards the directions in which single molecules are moving at any instant as determined casually, hence (in volumes and times exceeding a certain minimum) as radially symmetrical in the different directions of space; diffusion, the pressure exerted by gaseous and dissolved substances, the effects of temperature, entropy, are all expressions of this chance-determined symmetry. The constant rates shown by elementary physical and chemical processes under uniform conditions (kinetic or velocity constants) are based on this randomness, which is accordingly to be recognized as a general regularizing condition in nature. There is no satisfactory evidence that the physical law of automatic increase of entropy is permanently evaded in living organisms, although there is the possibility that such evasion may occur locally and temporarily in sufficiently small elements of volume. Transmission and amplification of these local effects may conceivably play an important part in irritable systems, such as the nervous systems of higher animals. This is a possibility which should not be overlooked;<sup>2</sup> but in general stability based on the omnipresence of random activities is as important in living organisms as in non-living systems.

In addition to stability of this randomly determined type, living organisms exhibit many stable features of

<sup>1</sup>Cf. E. S. Russell's book, "The Directiveness of Organic Activities," Cambridge University Press, 1945.

<sup>2</sup>Cf. my recent book, "General Biology and Philosophy of Organism," University of Chicago Press, 1945, p. 78.

structure and activity which are obviously based on the characteristic biological directiveness. Directive activity may be activity determined consciously in present time—*e.g.*, in a human being under the control of some purpose or motivation; or it may be activity determined by already existing physical structure which was originally laid down under directive control—*e.g.*, the case of machines. There is also the widely accepted theory that many organic structures and activities which now appear as directive have originated in evolution through the accumulation of favorable chance variations under natural selection; this is the Darwinian explanation of adaptation. Another view is that there may exist as a general condition running through nature a directiveness which is unconscious or subconscious.<sup>1</sup> But whatever its special origin may be, directiveness in living organisms is a general fact of observation which typically has its physiological basis in activities carried out under the control of definitely organized structure. Directive activities of this structurally determined kind may be called, “channeled,” or “canalized,” activities, and they form perhaps the most characteristic feature of living organisms.<sup>2</sup> Their application and direction are determined by stable routes or structures having definite position and orientation; in the adult animal these are represented by nerve-tracts, ducts, blood-vessels, muscles, tendons and other special features of organization. Channeling is also an essential feature in artificial machines, as well as in many of the external devices and constructions built by animals and even plants, such as nests, burrows, snares, seed capsules, galls, and so on. Random activities occurring in the interior of the channels may thus be given definite direction; but in

<sup>1</sup> The Hartmannian or Freudian “Unconscious” would come in here.

<sup>2</sup> I have discussed the subject of channeled activities in more detail in a paper entitled “Some Aspects of Theoretical Biology”, in *Philosophy of Science*, 1948, Vol. 15, p. 118. Channeling may be described as the guiding thread running through and unifying the mass of casually determined detail; as such it is a universal feature of vital organization.

themselves they remain random and the organism depends for its normal functioning on the regularity determined by this randomness; this is illustrated by the mixing processes in the intestine or vascular system which insure the uniform distribution of food-materials and oxygen. Randomness similar to that of mixing and shuffling operations also plays its part in many fundamental vital processes. The distribution of genes in fertilization, Mendelian inheritance and crossing-over is apparently determined by probability conditions. Similarly, in pure chemistry the constants of mass-action and reaction-velocity are statistically based and determined by the physical summation of random molecular motions.

The determinative importance of random activity at the molecular and subatomic levels is well recognized; but we must not overlook its presence in large-scale natural events also; all these, when attentively observed, are found to have in them a greater or less element of randomness. This is true also of conscious purposive activities, no matter how carefully they may be carried out. Put a glass on the table; it vibrates before it comes to rest; a wet sponge does the same, although the damping is more rapid. Any feat requiring special care, like replacing a galvanometer suspension, makes one vividly aware of the large element of contingency in all physical processes. Psychical events also appear as largely subject to chance: one forgets a name; later it floats up in memory; an idea comes unaccountably into one's head—or fails to come—and so on. Usually in purposive activity we ignore this casual element as far as possible, and pay attention only to the end in view and the conditions and actions needed to realize that end. But trivial occurrences continually remind us how largely the details of natural process are controlled by casual conditions. Undirected and undesired events occur with the same physical precision as those which are carefully directed. We realize this when the grapefruit spurt hits us accurately in the eye! Such

randomly determined events accompany every action; it is only when they may have important consequences that we pay any attention to them.

This omnipresence of contingency in nature has important biological implications. Somewhat paradoxically—as it may seem—it both makes directive action possible (as general physical stabiliser) and limits the possibilities of such action. In any voluntary action casual and directed events are always closely associated. Often we have difficulty in preventing casual events from taking the upper hand and controlling the whole situation, as in learning to skate or ride the bicycle. We have just seen that in many physiological processes the organism relies in chance-controlled conditions for stability. When the human being is acting in his directive or purposive capacity, his conscious volition may intervene only now and then, or perhaps only once at the beginning of the action, when the aim is to impart some guidance or integration to the chain of events. Usually those events which are under immediate directive control constitute only a small fraction of the whole nexus. When direction is applied only at the beginning of a complex sequence, the latter may appear to scientific examination as completely casual and physical. But at this beginning there may be a dichotomy of potential choice, alternative routes of activity being equally possible in the physical sense. One of these routes is determined, to the exclusion of the other, by the physical act of choice; the rest of the action follows automatically in accordance with the existing distribution of structural and randomly controlled physical factors. Consider the overwhelming preponderance of external casual factors when a ship is steered to its destination through a stormy sea; an occasional turn of the rudder is all that is needed to keep it to the desired course. Wind and wave in a hurricane show casual conditions at their worst; yet this does not prevent the directive agent from realizing his purpose; on the contrary, the very excess of randomness is

what makes the situation regular, and to that degree manageable.

From such considerations it seems a fair conclusion that the conjunction of casual and directive factors is a universal feature of natural events—although the ratio of directiveness to randomness may vary greatly in different instances. In the purposive actions of human beings this ratio is relatively high; in the events of inorganic nature (according to our usual conceptions of physical action) it is low or zero. But if the living is in fact an evolution from the non-living, it would seem to follow that the essential properties of the living must somehow be present in latent or undeveloped form in the non-living. Something corresponding to a directive factor would then be present and active everywhere in nature. This factor would assert itself in the midst of conditions largely or predominantly random. Nature is in fact not uniform in its structure but highly differentiated. According to Whitehead this inner element, which he calls "subjective aim," is an ingredient of all individualized systems or prehensions, *i.e.*, of all actual entities.<sup>1</sup> This element would correspond in a general way to what we are now calling the directive factor.

The microphysical conditions regarded as existing in a gas or solution may serve as a general illustration of how such a factor may act. At any instant each single molecule is regarded as moving in a definite direction and differently from most others, the directions and velocities of the motions being randomly distributed. Over the whole volume such actions are summed statistically so that the impact effect or pressure is everywhere the same. But suppose that all molecules were moving in the same direction at the same time—or even had a preponderant motion in one direction—there would then be something like a regimental effect, and the whole mass would move

<sup>1</sup>"Process and Reality," *passim*. In the most general sense, through the presence of this factor "there is initiated a definite outcome from a situation otherwise riddled with ambiguity" (p. 523).

forward in the direction of the prevailing motion. This is an imaginary illustration; as it is, the chance divergences cancel one another out and the result is a steady state of uniform pressure in all directions. If, however, we could introduce some directive influence pervading the whole volume and affecting uniformly all molecular motions, an asymmetrical activity such as we have imagined would be substituted for the symmetrical; such an effect might result, for example, in a gravitational or electrical field.

The asymmetrical action characteristic of living organisms appears, however, to be a small-scale rather than a large-scale effect, and to have its incidence in small groups of molecules or single molecules rather than throughout the whole system. It is a selective action, applied on the microphysical or molecular scale, as is shown most strikingly in the chemical reactions of constructive metabolism. The organism is built up and maintained by orderly combinations of complex organic reactions, and of these the most typical are asymmetrical. The classical illustration was given by Pasteur in 1860. Tartrates synthesized in the laboratory are optically inactive in solution, in contrast to the activity of natural tartrates; but Pasteur showed in his experiments with crystallization that this was because equal numbers of dextro- and laevo-molecules were produced. Living organisms are selective or preferential in their chemistry and synthesize or metabolize one form almost to the exclusion of the other. They act asymmetrically, or what might be called directionally. From this fact Pasteur drew far-reaching conclusions; he regarded this selective action as constituting an essential and profound difference between the chemistry of living organisms and that of non-living systems. We may consider this difference as ultimately responsible for the ability of living organisms to synthesize compounds of such extraordinary complexity. In the absence of any preference for asymmetrical modes of reaction, simple

types of polymerization might be possible, but hardly the construction of molecules like the proteins having a high degree of internal differentiation and an orderly arrangement of the component sub-molecules, resulting in a highly detailed specificity and antigenic property. Without some kind of directive control the random movements of the different amino-acid molecules would cancel one another out and lead to uniform instead of specifically ordered arrangements, resulting in simple structures of an aggregative type, as in other instances of haphazard mixing.

Evidently there is in living organisms a selective influence, acting on the micro-scale, which favors a preponderance of certain atomic motions in one direction; accordingly a laevo-tartrate or laevo-amino-acid is formed. We are now considering the simpler molecules, and not the complex structure of the native proteins showing a definite serial arrangement of the amino-acids and other sub-molecules. It is clear that whatever the influence may be which determines this type of asymmetrical molecular construction, it must be a persistent or sustained influence, otherwise the random or shuffling molecular motions always present would soon cause reversal of any special arrangements formed, and there would be no steady progress in a definite direction. Just this, however, is what is required to produce molecular structures with the complex and highly specific architecture which we find. How is this directive influence to be conceived as originating? And maintained after origination?

Since these synthetic reactions are presumably step-wise, and their details and end-results are constant, we must assume the existence of some constant determining condition converting motions that would otherwise be random into asymmetrical motions of a definite kind. One way of accounting for the asymmetry of biochemical syntheses is to refer them to other asymmetrical factors, such as asymmetrical catalysers, asymmetrical structural conditions, or asymmetrical influences from outside. As Japp

remarked in 1898, asymmetry can beget asymmetry.<sup>1</sup> This gives a regress, but not the original source of the asymmetry. Apparently we must refer back to asymmetrical conditions existing before the origin of living organisms, and there has been considerable speculation regarding such possibilities. In fact, asymmetrical conditions are found everywhere in nature; thus the earth rotates from west to east—not east to west—and the mechanical influence of this rotation (as seen in cyclones) differs in the two hemispheres; the conditions at a surface (*e.g.*, of the primordial sea-water) are different on the two sides; the solar radiation comes from one direction; moonlight is circularly polarized, and so on. Possible illustrations are innumerable; apparently any differentiation carries with it some kind of asymmetry or polarity, and it does not seem that randomness alone can ever account for the existing differentiation of nature. Reference has also been made to single chance factors; at a given instant any individual motion is in one direction, *i.e.*, asymmetrical in relation to physical nature as a whole. By some lucky accident of physical influence—radiational, electrical, mechanical—one or more asymmetrical organic molecules may have been formed; these may have determined (or catalyzed) the formation of others; one might suppose that molecular aggregates were thus formed having potential vital properties, such as power of reduplication based on autocatalysis, specific synthesis and growth, and self-protective responsiveness to environmental change.

It is, however, difficult to believe, when we consider the omnipresence of random activities in nature and the general opposition which they offer to complex differentiation, that a system originating in any such casual way could maintain and perpetuate itself, and later give rise to descendents forming an evolutionary line. Evidently

<sup>1</sup> F. R. Japp, Presidential Address, British Association, 1898 (published in *Nature*, 1898, Vol. 108, p 452).

the original living system, with its special powers of growth and reduplication, must have persisted and produced variants which also persisted. What actual conditions, existing constantly in nature, can we point to as furnishing the support for such a synthetic process?

This question brings up the consideration of causal and generative conditions in general, as we find them existing in nature. Summarizing briefly, we may say that in any single case of physical causation the determination of single events is ascribed to conditions already existing locally (*i.e.*, at some position in space) and having the general character of derivation from the past: temporal and spatial continuity between caused and causal conditions is assumed. The special conditions and factors present at the given space and time determine what happens at the spaces and times immediately adjacent: a thing acts when and where it *is*. But if this means the *exclusive* determination of an event by events and conditions immediately preceding in time and contiguous in space, it would hardly seem to meet the full requirements of the case, especially in living organisms. In particular, it accounts insufficiently for the fact of *novelty*. Laplace said: "the caused is predictable;" this statement asserts the complete regularity of causation and implies that both the past and the future of nature are equally calculable, given sufficient knowledge of present detail. But the production of novelty, or what is now called emergence, is a constant feature of natural process; routine emergent events (like the generation of water from hydrogen and oxygen) are not predictable until they have once happened; while certain complex kinds of emergents (like original works of art) appear as entirely unprecedented and creative. Emergence is best illustrated in conscious psychical phenomena; here we observe conditions arising in the present which have every appearance of being entirely novel and in large part independent of the past. The creative imagination is the best illustration, perhaps

the least predictable of natural processes. Evidently both kinds of emergence, routine and creative, have their importance for biology; but creative emergence is of special interest, since it is to be regarded as the ultimate source of novelty, and hence as underlying both individual creation and evolutionary progress.<sup>1</sup>

Evolution is an historical process and involves the progressive emergence of novelty. The historical past is to be regarded as largely—not entirely, for certain conditions are timeless or “primordial”—a left-over or remainder from activity which at one time was in the present. Nature is an advancing process, not merely a static condition and routine; there is always something incalculable in present events and the future which emerges from them. The present is that part of the temporal field of nature in which some element of innovation always adds its contribution to the total activity. As the present recedes into the past, the past conditions are altered and this alteration is permanent; the past is “irrevocable.” Here we have the universal natural fact of conservation; its corollary is that conditions once established persist through all time, including the present and the future (when it arrives); nature is essentially cumulative. The future, of course, is not yet existent; but all activity in the present adds something new to the sum-total of permanent natural conditions, and hence becomes a determinant in the possibilities of what will emerge later. Common sense as well as philosophy has always recognized this general fact of historical accumulation and determination; but the essential point now to be emphasized is that this historical conditioning does not exhaust all the possibilities of determination. Something must be attributed to spontaneous or “free” activity originating in the present; and the results of this present activity are seen not only in the unpredictable features

<sup>1</sup>I have discussed emergence more fully in the already cited paper in *Philosophy of Science*.

of individual action, but also in the special sequences of evolution, based as they are on individual variation.

Why is it necessary to emphasize this conclusion? The answer is obvious enough. The conception of complete causal determination by conditions already in existence—*i.e.*, by past and environmental conditions—was carried over from physics into biology, chiefly because of the scientific advances of the eighteenth and nineteenth centuries which demonstrated the fundamental importance of physical factors in vital processes. By an extrapolation the living organism was conceived as a purely physical system; as such it was regarded as rigidly deterministic or “mechanistic,” and any element of inner spontaneity or freedom seemed ruled out as physically impossible. This conclusion was extended by many to include human conduct; the element of internal or autonomous directiveness, originating in each individual in present time and apparently largely independent of the past, was regarded as unimportant or non-existent. This involved the denial of moral responsibility in the traditional sense. Such an inference ran counter to time-honoured human conviction, but to many it seemed logical and acceptable, especially among scientific men. At the present time, with our more adequate conceptions of physical causation, such a purely mechanistic interpretation is no longer scientifically acceptable; but some substitute must be found if we are to have a theoretical biology that will meet realistically and adequately all the problems of the living organism. Such an aim does not seem unrealisable if we recognize frankly that the vital process involves psychical as well physical factors. Directiveness is a universal character of living organisms; and, if we may judge from our immediate experience, it always includes some element of anticipation and subjective aim, involving future reference; these are characteristic features of the psychical. The psychical is foreseeing and integrative in its essential nature;

it tends to finish or round off an uncompleted experience.<sup>1</sup> To recognize this property as having its special importance in the living organism is not to ignore or undervalue the stable physical conditions which also form an indispensable part of the vital organization. In the psychophysical system which is the organism, factors of both kinds are to be regarded as equally important and as always supplementing one another in the total activity of the system.

The main question is: How are the biological facts of directiveness to be accounted for theoretically in a manner consistent with our knowledge of both the physical and the psychical aspects of the organism? Of primary importance is the fact that in addition to its special features as a sequence of physical events each organic directive action exhibits a well-defined unitary or integrated character. The minute physical details have no vital significance in themselves, but only as components of a process which is coherent and unified and typically leads to some future stage of importance to the organism-as-a-whole. On the psychical side it should further be noted that in any voluntary directive action only a small part of the demonstrable physical detail has its representation in the integrated consciousness. The subject is immediately aware of some desire, aim or purpose which maintains its essential character during the period occupied by the action. During this period the physical state and the activity of the system may change profoundly, and yet the guiding motivation persists, and has the appearance of acting as a regulatory factor holding the sequence of events to a definite course.

This unity has its close analogies to the unity which pervades the organism during its entire lifetime. As a model for this integrative agency or condition a comparison with the physical "field" has often been employed.

<sup>1</sup> A feature discussed by W. Köhler in his book, "The Place of Value in a World of Fact" (New York, Liveright Publishing Corp., 1938).

Iron filings suspended in a viscous medium and exposed to a magnetic field may take some time to assume their final regular configuration; but eventually they reach this state and maintain it, despite local and temporary disturbances. The formative influence pervades the whole space occupied by the system and imparts a definite arrangement to the otherwise incoherent particles. Obviously the living organism, considered as a composite of physical factors and events, is vastly more complex; at the same time its component processes, considered singly, are routine and automatic and are regulated by physical conditions, many of which are well understood. The most characteristic vital processes, organic synthesis and growth, have their purely physical side, and may proceed without any unifying directiveness being evident in the total result, as for example in tissue culture and abnormalities like tumors. Nevertheless, in normal ontogeny growth is a characteristically directive process which follows a definite course and ceases only when the adult stage is reached. Even then the cessation is only apparent, since organic maintenance cannot be regarded as differing essentially from growth.

The problem of the unifying factors in ontogeny is the biological problem of development, which is the same as the problem of heredity. According to modern genetics, the organismic unity has its physical basis in a definite biochemical and structural unity which pervades the entire organism at all stages. This is the gene system, located in the nuclear chromosomes, each of which has its special structure and biochemical composition, the latter consisting essentially in a polymerized combination of nucleic acid and specific protein. Apparently these nuclear units are the only organized units which have the same structure and composition everywhere in the organism at all stages. They may therefore be regarded as the physical correlate or representative of what we may call the "organismic field." The biological conception of field

is still in many ways a tentative one, always subject to the test of experiment. Its essential features may be briefly outlined as follows. The field is already present in the egg, and is to be conceived as expanding spatially with the growth of the embryo; in this process it becomes subdivided into secondary fields (organ fields) which often have considerable independence, as shown by transplantation experiments. The transplanted part may show autonomous growth on its own account; or it may influence specifically—or be influenced by—the region to which it is transplanted; this is the well-known “induction” or “organizer” effect, shown, *e.g.*, by the dorsal lip of the amphibian blastopore in initiating the formation of the embryonic nervous system. Many similar examples are known and much progress has been made in the physiological analysis of this fundamental biological condition. But the distinguishing character of the field is always the directive, integrative, or whole-making influence which it exercises within the organism.

In normal development and maintenance the secondary field influences are subordinated to those of the primary field, which extends over the whole organism and on its temporal side embraces the entire life-history. Apparently one manifestation of this influence is the maintenance of the personal characteristics of a human being during his lifetime. General facts of this kind are apparently without non-vital analogies, and they have led many biologists to emphasize the importance of non-physical factors in the total organismic integration; this is the case, for example, with Driesch, in essential agreement with Aristotle. As an example of primary integration, Gurwitsch cites the case of the developing mushroom: the hyphæ which build up the umbrella-shaped disc grow apparently at random in the interior of the structure, but change their course when they approach the surface, in such a manner that the regular arched and circular form is preserved. A purely physical interpre-

tation might seem possible in this instance (*cf.*, the effects of light and oxygen), but is difficult to conceive in others, especially in higher animals; for example, the participation of psychical factors is a demonstrable fact in the training and education of children. The collective influence of such factors may be regarded as corresponding to a field influence of a certain kind, but one which is primarily psychical rather than physical. This influence, it is perhaps needless to say, always manifests itself in association with physical factors acting as "efficient causes," *i.e.*, as factors concerned in the production and transfer of physical materials and energy. In the psychophysical system the psychical cannot be separated from the physical, although under some conditions it may play the dominant controlling part, as in the example just cited.

The field concept of the ontogenetic process has reference to a general biological condition, without giving any explanation of how the field originates or how its influence is exerted. This influence has been described as "regulatory;" regeneration is often called, "form-regulation," and as such is placed in the same category with other physiological regulations, in some of which the physiochemical basis is well understood, *e.g.*, the regulation of neutrality in the blood. The regulatory systems of the organism make their appearance in orderly sequence during ontogeny; they originate under the influence of the primary field as secondary mechanisms of special kinds. This is the same as saying that they are "inherited," and, as already remarked, the problem of inheritance is the fundamental problem of biology. Inheritance, as a complex reduplicative process, is necessarily directive, and manifests itself largely in the production of channeled activities and their subserving structures. But general biology seems to lack conceptions which are broad enough to provide a unitary and consistent theory of the general vital characters which are

common to the special facts of genetics and to those of the other departments of physiology.

The physical facts of genetics and cytology point definitely to the conclusion that the unifying or whole-making organismic influence asserts itself primarily through the cell-nuclei. Such a conclusion would refer the ultimate directive influence to intranuclear factors. But just what such a statement might mean realistically is not clear: does it mean that the unifying influence has its origin in physical or biochemical processes whose special locus is in the cell-nuclei (genes)? Or, alternatively, does it mean that its essential nature is other than purely physical and that it merely acts upon the physical system *through* the genes,—very much as a musician expresses himself through the keyboard? The problem of the nature of the biological nuclear influence is evidently fundamental, and one much discussed at the present time. On the biochemical side, the problem is concerned largely with the special properties of nucleic acid,<sup>1</sup> especially the desoxyribose form present in the chromosomes; why<sup>2</sup> is this compound a universal constituent of cell-nuclei and of self-proliferating quasi-living agents like viruses? What is its relation to the specific syntheses which are the essential feature of growth and maintenance? Its phosphate content is regarded as important in relation to energy-transfer (as also in muscular contraction), but this conception throws no light on the directiveness of the synthetic process. Simple reduplication of specific protein pattern may perhaps be accounted for by some form of the template or printing-press model, but this by itself seems hardly sufficient.<sup>3</sup> In any case, the special configuration and properties of the gene proteins in any species are to be referred, for their ultimate origin, to the evolutionary

<sup>1</sup> The Subject of the Symposium at Cold Spring Harbor during the summer of 1947.

<sup>2</sup> "Why", in the sense of referring to some more general determinative condition. But how far back does the regress have to go?

<sup>3</sup> Other possibilities are discussed in H. J. Muller's Pilgrim Trust Lecture, "The Gene" (Proc. Roy. Soc. B, 1947, Vol. 134, pp. 1-37. Cf. pp. 20-21.

history of the organism; in this process the progressive accumulation of genetic factors and their adjustment to the biological requirements of the whole organism represent the essential problems with which biological theory has to deal.

The general conception of biological directiveness is also descriptive rather than explanatory. No one can say "why" aim or direction should be an apparently universal feature of living organisms—and apparently also of the general natural process which is their background. Aim has the appearance of being itself a universal natural factor, a character of the creativity which according to Whitehead is ultimate<sup>1</sup>; it is a prerequisite of any novel determination, rather than something which is determined by conditions still more ultimate. The directive activities of the organism, when analysed physiologically, show nothing but sequences of physical and chemical processes. But in the more complex cases of directiveness, those of which we are immediately conscious, we are directly aware of the decisive part played by psychical factors as manifested in individual aim and effort. In every voluntary action there is present and observable the physical and physiological sequence of processes with its constant and causally interlocked conditions and factors; but the special course which the sequence follows is determined by the desire and purpose of the agent. The action itself, as a natural occurrence influencing other actions in the external world, is physical; and, as in other physical events, certain physical consequences persist and, as persistent, contribute to the general natural factors of conservation. A special form of conservation in living organisms is memory. The presence of conservative factors is seen in all constant and repetitive modes of natural action, whether physical or psychical; obviously, any kind of regularity is based on conservation. Novel action, however, requires in addition the entrance of factors of a non-conservative kind, and these are characteristic of

<sup>1</sup> "Process and Reality", *passim*: cf. pp. 11, 31 and following.

the psychical rather than the physical.

The general conclusion follows that in any instance of directive action two equally fundamental conditions or determining sets of factors are to be distinguished; one (A) is the factor of conservation corresponding to the established physical order lying behind all regular causation, and having its special exemplification in the action under consideration. The other (B) is the factor of *aim*, with its features of anticipation and novelty, existing in the present but with a future reference, and having the special inner self-determining property which appears to observation as psychical. As already indicated, the physical background is always actual and continuant through past time up to the present; in contrast, the psychical factor has its existence and activity only in the present with its brief and variable time-span. Conceivably the psychical need interpose its influence only for a brief interval during this present, at some critical locality or intersection of events, in order to change the whole course of the physical sequence which is determined at that locality. A dichotomy of possible action, *e.g.*, at some region in the cerebral cortex where the spatial direction taken by some microphysical energy-exchange depends on the choice made by the psychical factor, seems to be all the condition that is required for directive control.<sup>1</sup> The nervous currents thus started along definite structural pathways travel automatically to their terminal muscle-groups—unless deflected or inhibited by other influences (nervous, chemical, mechanical) met either *en route* or at the regions of final distribution. The general condition thus described would admit of endless variation in detail, according to the special structural arrangements of the organism, or the special physical and psychical influences acting within it. It should also be remembered that physical amplification is always a factor in processes of stimulation—which are electrically con-

<sup>1</sup> One might think of a double-throw electric switch as a large-scale analogy.

trolled processes—and this factor may be assumed to play a part in all forms of nervously controlled activity. Hence a large effect may result from a local process originating in a microscopic or ultra-microscopic area.<sup>1</sup> While I am now referring specifically to nervous action, because of its demonstrable association with directive processes in higher animals, considerations of a similar kind (*mutatis mutandis*) apply to other physiological processes involving stimulation and response. Not only individual conscious behaviour, but many organic activities of the subconscious or unconscious class may also be regarded as subject to directive control, including, ultimately, developmental processes and germinal mutation. Unfortunately, direct evidence is inaccessible in these cases; they belong in the class of “unobservables.” It is well to realize, however, that if we accept the psychophysical conception of the organism, a directive control of mutation and ultimately of evolution remains no longer physically inconceivable. Certainly evolution, both inorganic and organic, has followed a path that can hardly be ascribed entirely to the play of random factors. This special conclusion may be regarded as merely an expression of my personal conviction; but in a universe where most of the detail is unobservable, the application of well-supported general principle to particular instances is all that may for the time be possible. That the living organism is a psychophysical system is undoubtedly an empirically well-supported principle.

<sup>1</sup> “General Biology and Philosophy of Organism”, p. 78.

## SYMPOSIUM ON PLASMAGENES, GENES AND CHARACTERS IN *PARAMECIUM AURELIA*<sup>1</sup>

### INTRODUCTION<sup>2</sup>

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The interrelations between genes, cytoplasmic factors (plasmagenes) and traits in *Paramecium aurelia* are under investigation by a corps of workers in several laboratories, with major discoveries emerging at so rapid a rate that there is need periodically to synthesize the results and see where they are heading. This symposium is an attempt to present the current status of these investigations. The purpose of this introductory paper is to provide the background for the special papers that follow and to integrate them into one general picture. The program falls into three parts, each dealing with a different trait from the common points of view. Part I of this introduction should be read before the first four papers on the "killer" character; Part II after these and before the next two papers on antigenic traits; Part III after these and before the last paper on mating types.

#### I

The killer character dealt with in the first four papers (Preer, 1948; Dippell, 1948; Austin, 1948; van Wagten-den, 1948) is the one which has been most fully analyzed from all three points of view: gene, cytoplasmic factor and trait. Certain strains of paramecium, called killers, liberate into the fluid in which they live a poison, "paramecin," which kills paramecia of other strains

<sup>1</sup> Introduction to Symposium on this subject presented before a joint meeting of the American Society of Zoologists and the Genetics Society of America at the Chicago meeting of the American Association for the Advancement of Science, December 29, 1947. Contribution No. 380 from the Department of Zoology, Indiana University.

<sup>2</sup> The previously unreported experimental work described in this paper was aided by grants from the Rockefeller Foundation and Indiana University.

known as "sensitives" (Sonneborn, 1939, 1943). Killers are resistant to their own paramecin; indeed they normally carry some of it within their bodies (Sonneborn, Jacobson and Dippell, 1946). The production and liberation of paramecin, as well as resistance to its action, depend upon the presence in the killers of a cytoplasmic factor, "kappa" (Sonneborn, 1943, 1945b). Although kappa multiplies, its maintenance and increase depend upon the presence in the nucleus of a dominant gene, K. If this gene is replaced by its recessive allele, k, kappa completely disappears and cannot be again produced even if the dominant K gene is restored. Hence, K cannot initiate the production of kappa. In the absence of kappa, regardless of whether K or k is present, the animals are not killers, *i. e.*, they cannot produce paramecin; they are, in fact, sensitive to it.

This remarkable dependence of a trait on a semi-autonomous, multiplying cytoplasmic factor which cannot be gene-initiated calls for investigation in great detail. In such a study, one needs to discover in full the nature and properties both of kappa and paramecin. The properties of kappa are dealt with in the first two papers (Preer, 1948; Dippell, 1948). Dr. Preer reports the number of kappa particles in a killer, their rate of multiplication, and their size. Miss Dippell presents the proof that kappa is capable of mutation. The other two papers of this group (Austin, 1948; van Wagtendonk, 1948) discuss the properties of the poison, paramecin, the production of which is controlled by kappa. Professor Austin presents the evidence demonstrating that a sensitive animal may be killed if it takes up one particle of paramecin, and shows that paramecin is liberated under certain conditions at the rate of one particle per killer animal per five hours. Professor van Wagtendonk presents the results of a chemical study of paramecin, indicating that it is a desoxyribonucleoprotein.

## II

Professor Austin's account of the activity of single particles of paramecin and Professor van Wagtendonk's evidence for the desoxyribonucleoprotein nature of paramecin both raise the question of whether paramecin itself may be a multiplying particle similar to, or perhaps even identical with, kappa. However, deliberate efforts to detect multiplication of paramecin have thus far invariably failed. Sensitive animals attacked by paramecin liberate no paramecin when they die and disintegrate, nor do they yield any detectible paramecin when their bodies are broken up experimentally at any time after exposure to paramecin. Other observations weigh against identifying paramecin with kappa. For example, Preer (1946, 1948) estimates 200 to 1600 kappa particles in a killer animal, a figure enormously greater than the estimate of 3 to 4 particles of paramecin in a killer given by Sonneborn, Jacobson and Dippell (1946). Moreover, when the kappa concentration is reduced by exposing killers to high temperatures (Sonneborn, 1946), their bodies contain no detectible paramecin. That paramecin is absent from such animals is further indicated by the fact that they live and multiply although they are readily killed if exposed to paramecin. Unless one assumes that paramecin is harmless inside the body of a sensitive animal, it must be concluded that kappa-bearing sensitives contain no paramecin and, therefore, that kappa and paramecin are not identical. In view of these and other facts, one cannot at present identify kappa with paramecin or demonstrate that paramecin multiplies. Further and decisive evidence on these problems is, however, much needed.

Other questions arise from the studies of Dr. Preer and Miss Dippell on kappa. They show that the properties of kappa are similar to those of a gene: both affect a trait, both multiply, both are mutable. Indeed, it has previously been suggested (Sonneborn, 1945a, 1946) that kappa might be descended from part of a more complex ancestral

K gene. Preer's new evidence on the size of kappa indicates it to be far too large to be derived from a gene.

What then is kappa? Is it a virus or "viroid," as has been suggested (Lindegren, 1946; Spiegelman, 1946; Altenburg, 1946)? The properties which kappa shares with genes it also shares with viruses; and its size falls within the range of the larger viruses, rickettsias and small bacteria, as Preer (1948) points out. Further, a host can often be "cured" of a virus and both Preer (1946) and Sonneborn (1946) have shown that killers can be "cured" of kappa, *i.e.*, they can be freed of it.

Thus far, one of the main distinctions between kappa and a virus has been with respect to the mode of transmission. Kappa is transmitted by heredity, it is passed on from parent cell to daughter cell, whereas viruses are transmitted by infection. During the past four years, numerous attempts have been made to transmit kappa by infection, but they have invariably failed. Even this distinction between kappa and a virus seems now to have disappeared. I have recently succeeded in transforming sensitives of the proper genotype (K) into hereditary killers by exposing them to concentrated suspensions of the broken-up bodies of killer animals. Previous failures were apparently due to the use of insufficiently concentrated suspensions. The suspensions most active in transforming sensitives into killers are those just below a concentration that kills the animals (not by paramycin action) within an hour or two; they contain broken-up bodies of up to 100,000 killers per cubic centimeter. One or a small number of sensitives is introduced into each 0.1 cc. sample of the suspension. Many of the sensitives are killed by the paramycin in the fluid and all take up paramycin; but a considerable proportion produce viable descendants for a large proportion of animals attacked by paramycin produce viable descendants if an excess of food is present (Sonneborn, Jacobson and Dippell, 1946). After the survivors exhaust their food supply, they are provided with only enough food to permit them

to undergo one fission per day. Under these conditions, even if only a single particle of kappa is initially present, it will in the course of several days accumulate to a concentration sufficient to control paramecin production (Preer, 1946). In the most successful experiments, up to 50 per cent of the exposed sensitive animals that produced survivors yielded a culture of hereditary killers. The simplest interpretation of these results is that kappa, released from the broken-up killers, entered the bodies of the sensitives where it multiplied and transformed them into hereditary killers. The detailed data suggest, moreover, that even under optimal conditions only one or a few of the millions of kappa particles present entered and multiplied in any one sensitive paramecium.

These new results thus lead to the conclusion that kappa cannot be distinguished from a virus even on the grounds of mode of transmission. On the other hand, similar infective transmission of transforming principles has been reported in bacteria by Avery and co-workers (1944) and by Boivin and co-workers (1945, 1946, 1947), and in *Drosophila* by L'Heritiér and Scoeux (1946), yet this fact has not seemed sufficient to warrant concluding that the transforming agents are viruses. In the bacteria they are believed to be specific desoxyribonucleic acids, a fact of much interest in relation to van Wagendonk's studies (1948) on paramecin. Darlington (1944) and L'Heritiér and co-workers (1945, 1946) maintain, after detailed consideration of the whole situation, that no fundamental distinction between plasmagenes and viruses is available. How these transforming and multiplying agents are to be classified seems to be a matter of arbitrary definition.

What these agents are to be called seems less important than obtaining an understanding of their nature and properties, such as is provided by the work of Preer, Dippell and ourselves, and obtaining evidence as to whether they are normal and integral parts of the genetic system. In order to answer the latter question, one needs to compare

the genetic system controlling the killer character with the genetic system controlling normal traits of the organism. The two papers by Sonneborn and Arlene LeSeur (1948) and by Kimball (1948) deal from this point of view with an unquestionably normal group of traits which all paramecia have, namely, antigenic traits. The first of these papers deals with antigenic traits that are cytoplasmically determined and inherited, the second with similar traits controlled by genes through the mediation of cytoplasmic factors. These two different modes of control and inheritance characterize different so-called "varieties" of *Paramecium aurelia* (Sonneborn, 1945a).

### III

The two papers on antigenic traits (Sonneborn and LeSeur, 1948; Kimball, 1948) support the view that the cytoplasmic factors or plasmagenes of *Paramecium* are, unlike viruses, normal and integral parts of the genetic system. These normal traits are determined and inherited through plasmagenes in variety 4, and Kimball holds they are mediated by plasmagenes in variety 1. Before discussing the general bearing of these results, however, there should be before us the main facts as to the determination and inheritance of another unquestionably normal trait, mating type, which is discussed from another point of view in the concluding paper by Dr. C. B. Metz (1948).

In variety 4 of *P. aurelia* the two mating types are known as VII and VIII. Ordinarily any clone of variety 4 is phenotypically pure for one of these two mating types. When the two types mate, the results as to inheritance depend upon whether cytoplasmic transfer occurs during conjugation. In the absence of cytoplasmic transfer, the type VII conjugant of each mated pair produces a type VII clone, the type VIII conjugant a type VIII clone. This indication of cytoplasmic determination is confirmed by the results observed when massive exchange of cytoplasm takes place between mates during conjugation. Under these conditions, both members of a pair of conju-

gants usually produce clones of mating type VIII, indicating that a plasmagene for type VIII has passed from the type VIII conjugant into its type VII mate. Sometimes, however, both mates of a conjugant pair produce clones of mating type VII. More commonly, one or both mates produce mixed clones including some lines of descent of type VII, other lines of descent of type VIII. This segregation of diverse hereditary types within one clone is further evidence of cytoplasmic determination, for all members of the clone have the same genic constitution. The fact that cytoplasmic exchange at conjugation sometimes results in a type VII clone from the type VIII mate and sometimes in a type VIII clone from the type VII mate suggests that there are two alternative plasmagenes, one controlling type VII and one controlling type VIII. Similar rules of inheritance hold for the mating types III and IV in variety 2, types XI and XII in variety 6 and types XV and XVI in variety 8, *i.e.*, in all of the so-called group B varieties (Sonneborn and Dippell, 1946).

The data just set forth for mating types and the data of Sonneborn and LeSuer (1948) on antigenic traits indicate that plasmagenes are involved in the determination and inheritance of these normal traits in this material. On the other hand, the question of whether the plasmagenes are gene-initiated is still open. In the work on kappa, the inability of the genes to initiate kappa was demonstrated by removing kappa and showing that it was never thereafter produced. The same type of analysis needs to be applied to antigenic traits and mating types. Thus far it has not been reported and, until it has, the question must remain open. The results presented in the two papers on antigenic traits show the dangers and difficulties besetting such an analysis. Kimball's data show how long a trait may disappear before gene-initiated plasmagenes bring it back to phenotypic expression. The fact that it always comes back eventually, together with genetic analysis, is evidence for gene-initiation of the controlling plasmagene.

In the different variety investigated by Sonneborn and LeSuer, similar traits may disappear permanently, yet these authors have shown the plasmatic basis of the trait is always present. Without such analysis, it would have been easy to fall into the error of concluding that the determining plasmagene had disappeared and that the genes were incapable of initiating it. But with their analysis, apparent loss of the plasmagene seems to be an illusion. Under such conditions it is difficult to get a clear answer to the question of whether or not the genes initiate the antigenic plasmagenes. Similar difficulties beset the question in relation to the mating type plasmagenes. Neither mating type reproduces absolutely true to type: at autogamy, a type VIII clone produces a type VII clone once in about 50 times and a type VII clone produces a type VIII clone once in several hundred times. Are these "mutations" like the antigenic "mutations" of Sonneborn and LeSuer, *i.e.*, shifts from one stable proportion of determiners to another? If so, then all paramecia of variety 4 contain plasmagenes for both mating types (VII and VIII) at all times and the question of their gene initiation cannot yet be answered.

Further understanding of the genetic mechanisms controlling mating types may therefore have to come from other than genetic methods of analysis, just as solution of similar problems in the case of antigens seems to depend upon other (immunological) methods of study. A method of studying mating types is now in process of development by Dr. C. B. Metz whose results, also of much interest in other connections, are presented as the last paper of this symposium.

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THE KILLER CYTOPLASMIC FACTOR KAPPA  
ITS RATE OF REPRODUCTION, THE NUMBER OF  
PARTICLES PER CELL, AND ITS SIZE

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WHEN killers of variety 2 of *P. aurelia* reproduce rapidly by fission, they increase in number faster than the cytoplasmic factor kappa. The number of particles of kappa per animal decreases until finally it reaches zero in some individuals. When animals with a decreased number of particles, but not completely lacking them, reproduce slowly the number of particles of kappa increases faster than the number of animals until the original concentration is restored. These changes in concentration of kappa are accompanied by both quantitative and qualitative changes in the killer character.

Control of the concentration of kappa by taking advantage of this relation between the rate of multiplication of the animals and the rate of increase of kappa has provided a technique for investigating certain properties of kappa. Three groups of experiments have been performed utilizing this technique and it has been possible to demonstrate (Preer 1946, 1948): (1) The capacity of a single particle to restore, under appropriate conditions, the normal concentration; (2) the number of particles per animal and their rate of increase; (3) the rate of increase of particles under various conditions. More recent work, reported for the first time in this paper, provides information concerning the size of kappa. A preliminary report of cytological investigations of kappa is also given.

The existence of this relation between the rate of increase of animals and kappa is shown by the following observations. Killers cultured slowly by providing a

small daily supply of food remain strong killers. But when strong killers are cultured rapidly by providing a large daily supply of food, they go through a series of quantitative and qualitative changes. First, the strength of killing gradually diminishes. Then animals which are non-killers, yet resistant to paramecin, are produced. This is followed by weak sensitivity to paramecin, and finally, in certain killer stocks, by strong sensitivity. These changes proceed more rapidly at very fast fission rates than at only moderately rapid rates. If animals which have been rendered non-killers by culturing rapidly are allowed to reproduce at a slow fission rate, the animals undergo the same qualitative and quantitative changes in the reverse direction and gradually become strong killers again. The longer the period of rapid reproduction has been and the faster the rate of reproduction during this period, the longer the period of slow reproduction required for the reappearance of strong killers. Finally, if the rapid fissions continue long enough and fast enough, the killer character is permanently lost, and animals remain sensitives under all conditions of growth.

The only reasonable explanation for these facts is that the rate of increase of the hereditary basis for killing, kappa, fails to keep pace with the rate of increase of the animals; the amount of kappa per animal is progressively reduced in quantity by successive fissions, and finally animals are produced which have no kappa at all. When animals containing a reduced number of particles of kappa are cultured slowly, kappa increases faster than the animals until the original concentration is restored.

Let us now turn to the properties of kappa which have been studied in experiments that utilized this relation between the rate of increase of kappa and the fission rate of the animals. In the first group of experiments it was possible to determine that a single particle of kappa in an animal is sufficient to enable that animal to produce

progeny with the normal particle number when reproduction is slowed. Such experiments were designed as follows:

From an original killer culture, which had been reproducing rapidly (and, hence, had been experiencing a decline in its kappa concentration), a number of animals were isolated, each one into a separate container. Each of these isolated animals was allowed to continue its rapid multiplication for a limited period of time, thus further reducing the kappa concentration. As all of the progeny produced from any one isolated animal were kept together in one container, if the isolated animal possessed even a single particle of kappa, then some of its progeny in the container must also possess kappa, providing no kappa is destroyed. Whether killer animals can arise among the progeny (when their reproduction is slowed or stopped, so that kappa concentration can again rise), depends on the minimum number of particles of kappa required for regeneration of the original kappa concentration.

If one particle is enough, then every animal that contained at least one particle of kappa when isolated will yield some killers among its progeny. The percentage of isolated animals whose progeny could experience regeneration of kappa would be completely independent of the duration of the rapid fissions after isolation. The progressive lowering of mean kappa concentration subsequent to isolation, though it occurs, is here irrelevant; the decisive fact is simply whether an animal in the culture contains at least one particle of kappa.

On the other hand, if regeneration of kappa is blocked unless *more* than one particle is present in an animal, then some animals which had only the minimum (or somewhat more than the minimum) number of particles of kappa when isolated would yield progeny none of which contained the minimum number of particles. This is true because, as just pointed out, the mean kappa con-

centration is further reduced during the period of rapid fissions following isolation. Under such conditions, therefore, the percentage of isolated animals whose progeny could experience regeneration of kappa would decrease as the period of rapid fissions is prolonged, for more and more would have their kappa concentration brought below the assumed minimum for regeneration.

Therefore, if one particle is sufficient, the percentage of isolated animals producing killer progeny would be independent of the number of fissions following isolation; and if more than one particle is required, the percentage of animals producing killer progeny should decrease with the number of rapid fissions subsequent to isolation. Experiments performed to test this point have revealed that the percentage of isolations giving rise to killer progeny is independent of the number of rapid fissions following isolation. This clearly shows that a single particle of kappa is sufficient to enable an animal under the proper conditions to give rise to progeny with the normal number of kappa particles.

The second group of experiments concerned with the properties of kappa will now be discussed. Here the number of particles of kappa in a killer and the rate of increase of kappa were estimated from the proportion of the progeny of a strong killer freed of kappa after different numbers of rapid fissions. In one such experiment on the killer stock G, a single killer was first isolated into a container and allowed to reproduce by rapid fissions. As the rapid fissions continued, the number of particles of kappa per animal steadily decreased, until finally some animals were produced which had lost all particles of kappa. Additional fissions caused an increasing proportion of the progeny of the original killer to lack kappa completely. The proportion of the animals lacking kappa after different numbers of fissions was determined by isolating series of animals at different times, culturing them slowly, and later finding

what proportion of the individuals isolated at any time gave rise to *no* killer progeny. As we have seen, these are the animals which contained *no* particles of kappa.

In order to calculate the number of particles of kappa in the original killer and the rate of increase of the particles, it is necessary to make certain assumptions. The chief assumptions are that particles increase by doubling at a constant rate, and that they are segregated at random to the two animals produced at each fission. A simple approximation of the number of particles and their rate of increase can be made by using the Poisson distribution to relate the proportion of the animals with no particles at any time to the average number of particles per animal in the culture at that time. By multiplying the average number of particles by the number of animals in the culture we can get the total number of particles in the culture. After determining the number of particles of kappa in the culture in this manner at two successive times, we can calculate the rate of increase of kappa. It was found that while the animals increase at a rate of about three and one-half fissions per day, the particles of kappa increase at a rate of about two doublings per day. Knowing the number of kappa particles at some one time and knowing the rate of increase of kappa, it is easy to calculate what the average number of particles and the proportion of animals with no particles at *any* time should be. Such calculated proportions agree with experimentally determined proportions, thus supporting the validity of the assumptions. When the average number of particles at the zero fission time is calculated, the number of particles in the single killer used to start the experiment is obtained.

For reasons which time does not allow me to discuss, this method of calculating particle number is somewhat inaccurate. Better methods of calculation, including a general mathematical treatment worked out by Richard

Otter of Princeton, indicate that there are about 200-300 particles of kappa in a strong killer. Certain considerations regarding the assumptions, however, make it seem probable that this estimate is somewhat too low.

A third series of experiments dealt with the rate of increase of kappa under various conditions of kappa concentration, fission rate and temperature. It was shown that the rate of increase of kappa varies with the kappa concentration. Kappa increases faster when in low concentration than it does when in high concentration. The rate of increase of kappa is closely correlated with the rate of fission; the higher the fission rate, the faster the rate of increase of kappa—up to a certain critical fission rate at which the rate of kappa increase reaches a maximum. As the fission rate is increased beyond this critical rate, the rate of increase of kappa remains essentially constant. Stock G kappa increases at this maximum rate of two doublings per day at about 27° C, the rate being less above and below this temperature. Between 29.4° C and 30.4° C and at higher temperatures stock G kappa is inactivated and gradually disappears from animals, even when they are not multiplying.

At present an attempt is being made to estimate the size of kappa through X-ray inactivation. When animals in killer cultures were radiated with doses of X-rays ranging from 19,000 *r* to 36,000 *r*, increasing proportions of the animals became permanently sensitive and produced only sensitive progeny. After doses higher than 36,000 *r* all animals produced exclusively permanent non-killer sensitive progeny. It is concluded that kappa's reproductive capacity is destroyed by the radiation. Since the radiation of viruses and bacteria gives inactivation due chiefly to the direct effect of the radiation, it is likely that the effect on kappa also is direct. And since the inactivation dose of viruses (the dose required to inactivate all except 37% of the virus particles) is inversely

correlated with size, it is likely that the inactivation dose of kappa also indicates kappa size.

The inactivation dose of kappa and also the original kappa concentration was determined as follows. Since animals containing at least one particle of kappa are known to produce some killer progeny, irradiated killers producing no killer progeny must have contained no active kappa. From the proportion of animals containing no kappa after different amounts of radiation, the number of surviving kappa particles was calculated by use of the Poisson distribution. Plotting logarithmically these average particle numbers against dose and extrapolating the resulting straight line, the original particle number for the killer stock G was determined as 400-1600. This particle number agrees reasonably well with the estimate of 200-300 made by differential growth rate studies, since, as already pointed out, the figure of 200-300 was thought to be somewhat too low. The point on the curve which gives a mean kappa concentration of 37 per cent of the initial concentration gives the inactivation dose. This dose can be read from the curve and is found to be approximately 3500 *r*. This dose is typical of that found for many bacteria and cells of somewhat larger size. Shope rabbit fibroma virus has an inactivation dose of 8000 *r*, and other large viruses range from 40,000 upward. Therefore, it is probable that kappa is at least as large as the largest viruses (0.1-0.3  $\mu$ ) or bacteria (0.15-3  $\mu$ ). Particles of this size should be visible with the compound microscope.

Cytological investigations are now being made in an attempt to see kappa and study its properties cyto-chemically. Preliminary studies reveal that the cytoplasm of most, and possibly all, of the killer stocks contain Feulgen positive particles. Feulgen positive material has not been found in the cytoplasm of the sensitive stocks thus far investigated. The particles found in killers of stock G are approximately 1000-2000 in number. They are less

than one micron, probably about 0.2-0.5 microns in diameter. The possibility that these particles represent kappa, is being investigated.

In concluding, four of the main points of significance of the work can be pointed out. *First*, it has been shown that the concentration of self-duplicating constituents may rise or fall in dependence upon whether the cells multiply less or more rapidly than the cellular constituent itself. *Second*, the cellular concentration of cytoplasmic constituents determines the character of the cell; properties that appear when their concentration is high, weaken and disappear as their concentration decreases. *Third*, the mathematical techniques employed, especially those developed by Otter have provided a general method of dealing with the distribution of self-duplicating cytoplasmic components in the course of cellular divisions. *Fourth*, the methods and results suggest applications to situations where sexual reproduction does not occur, such as the genetical problems of cell differentiation in embryological development and the genetics of asexually reproducing organisms.

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MUTATIONS OF THE KILLER PLASMAGENE,  
KAPPA, IN VARIETY 4 OF *PARAMECIUM*  
*AURELIA*

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In *Paramecium aurelia*, variety 4, the cytoplasmic factor, kappa, that controls the killer character is similar to a gene in some fundamental respects, but the basic gene property of mutability has not yet been analyzed for kappa, although indications of the occurrence of kappa mutations have been reported by Sonneborn (1946). Recently, spontaneous mutations of the killer character of race 51, variety 4, have been discovered and have provided an opportunity for determining whether the new characters are due to gene or cytoplasmic factor mutations, or both. This paper briefly summarizes the results thus far obtained.

Animals of killer race 51 produce in the fluid in which they live a toxic substance known as paramecin. Killers are resistant to their own paramecin, but sensitive animals exposed to the killer substance undergo a series of changes as shown in Figure 1, resulting in the production of blisters, a conspicuous posterior hump and, finally, death of the affected animals. Disintegration of the rounded corpses, illustrated by the last diagram, does not occur before 24 hours from time of exposure of animals to paramecin.

For the purpose of clarity, this type of killing will be referred to as "original" killing, and the killer gene, kappa, and paramecin will be designated as "original" killer gene, "original" kappa, and "original" paramecin. One of the mutant killers retains a similar type of killing

<sup>1</sup> Florence R. Sabin Fellow of the American Association of University Women. Aided by grants for research in genetics at Indiana University from the Rockefeller Foundation and Indiana University.

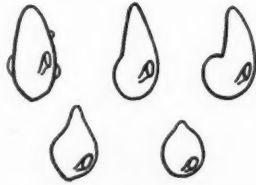


Figure 1. The series of morphological changes preceding death when sensitive animals of variety 4, *P. aurelia*, are exposed to paramecin of killer race 51. action but consistently produces less paramecin or less effective paramecin. Three other mutants produce an entirely different kind of killing effect which causes sensitives to spin violently on their longitudinal axis and finally to sink as if paralyzed to the bottom of the culture dish where they often become vacuolated before death. This mutant kind of killing action, under optimal conditions, is more rapid than that of the original (unmutated) killer, the sensitives often dying and disintegrating within eight hours after exposure to paramecin produced by the mutant killer.

Since the same sort of analysis was conducted for each of the mutants and since all gave essentially the same results, this report will be confined to an account of a mutant which kills sensitives in the spinning manner. This mutant is not only resistant to the paramecin produced by the original killer, but the paramecin of the mutant kills the original killer.

The first step in the analysis was to determine whether the mutant killing was dependent on a nuclear gene allelic to that gene, called K, which controls the maintenance of original kappa. This was done by crossing the mutant to a sensitive culture homozygous for the recessive allele k, which cannot maintain original kappa.

When such a cross is made, the killer conjugant of each mated pair yields a killer culture and the sensitive conjugant yields a sensitive culture (Figure 2, F1); both conjugants of each pair, however, are now genically alike and are heterozygous. If autogamy is induced in a cul-

ture of animals from the F1 killer conjugant, all of the autogamous animals (the F2 generation) will be homozygous. At autogamy two identical, haploid, gamete nuclei in an unmated animal unite to produce a diploid fertilization nucleus from which the new macronuclei and micronuclei are derived. Hence, all animals which have undergone autogamy are necessarily homozygous for all their genes. Since it is a matter of chance which genes, the ones from the killer parent or the ones from the sensitive parent, will be involved in this self-fertilization, 50 per cent of those animals isolated in the F2 will be homozygous for the recessive *k* gene. If the mutant killing action is dependent on the presence of the dominant allele, *K*, then its replacement by the recessive allele should result in sensitivity. On the other hand, the remaining 50 per cent of the autogamous F2 animals should be homozygous for the gene from the mutant killer and, hence, should show mutant killing as before. The results obtained when the F2 was tested (Figure 2) showed 57 sensitive lines to 59 killer lines of the mutant type, a close

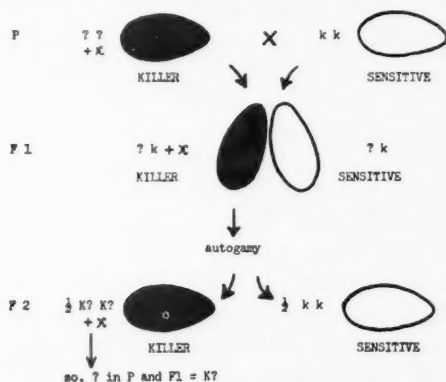


Figure 2. Cross to demonstrate the existence, in the mutant, of a dominant killer gene at the same locus as gene *K* in the original killer. Killer genotype of parent mutant equals (??) plus kappa. Parent sensitive contains the recessive allele of the original killer gene and lacks kappa. F2 killer shows genotype as determined from final result of cross.

approximation to be expected 1:1 ratio. This experiment shows, then, that a dominant gene at the K locus is necessary for the maintenance of the mutant killer character.

Whether or not this killer gene in the mutant is like the one in the original killer was determined by crossing the mutant to a sensitive culture containing the original killer gene K but lacking kappa (Figure 3).

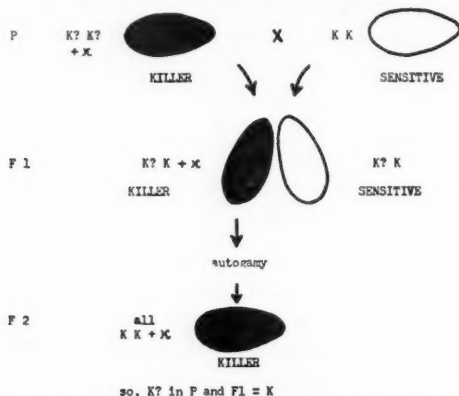


Figure 3. Cross to show killer gene in mutant and in original killer are identical. Killer genotype of parent mutant equals (K?K?) plus kappa. Parent sensitive contains original killer gene but lacks kappa. F2 killer shows genotype as determined from final result of cross.

The F1 mutant killer now contains one of its own killer genes (K?) and one derived from the original killer. If autogamy is induced in such an F1 killer culture, 50 per cent of the autogamous F2 should be homozygous for the killer gene from the mutant animal and 50 per cent homozygous for the original killer gene, K. Those homozygous for the killer gene from the mutant should remain mutant killers. On the other hand, if the killer gene in the mutant differs from that in the original animal and if this difference alone is responsible for the mutant character, then the 50 per cent of the F2 homozygous for the original killer gene should become original killers. The fact that 100 per cent of the F2 were mutant killers and that this

type of killing has persisted through at least two autogamies, shows that the original killer gene can replace the killer gene in the mutant and the mutant type of killing is still retained. Hence, the killer gene in the mutant and in the original killer must be alike as indicated in the F<sub>2</sub>, Figure 3.

The third step in the analysis was to determine if the killer gene in the mutant could maintain original kappa and if any locus other than the K locus in the mutant differed from the original in effect on kappa. The mutant was crossed to an original killer and an autogamous F<sub>2</sub> obtained from the clone descended from the original, not the mutant, member of the F<sub>1</sub> pair of conjugants. The results are still incomplete but they indicate that the killer gene in the mutant can maintain original kappa with original type of killing and that no other locus in the mutant differs from the original with respect to kappa control. Apparently, then, no mutation of the killer gene or any other gene has occurred to account for the existence of the mutant killer.

That the new character is due to a mutation of kappa, however, is indicated by two main types of evidence. First, the mutant analyzed above was found to contain two types of kappa. This was discovered by reducing the concentration of kappa to one particle per cell and then allowing it to accumulate in the cell according to the following method: Sonneborn (unpublished) has found that at 33.8° C., multiplication of original kappa is effectively stopped while the animals continue to divide at a normal rate. As a result, the cellular concentration of kappa is rapidly reduced, as in Preer's work (1946), until one obtains some animals having only a single particle of kappa. This single particle was allowed to build up to a maximal cellular concentration by restoring the animals to 27° C. (the usual cultivation temperature) and limiting their food supply so as to force the animals to multiply more slowly than kappa. When a single animal was

allowed to multiply at 33.8° C. and its progeny isolated and treated as above, some of the progeny were found to be pure for a kappa which produced paramecin of the "spinner" type. This kappa was called m2 kappa. Other animals were pure for a kappa which produced paramecin with effects identical with those of the original but the amount of detectible paramecin was extremely small. This type of kappa was designated as m5 kappa. The mixed mutant showed only one type of killing because the m2 kappa acts more rapidly than m5 kappa and overshadows the effect of the latter. The isolation, from one animal, of kappas with diverse killing action is strong evidence that the mutant killing is a result of kappa mutation.

Supporting evidence is obtained from the fact that each of these diverse types of kappa can be transferred from one conjugant to another in cases where cytoplasmic bridges are formed at conjugation. Some pairs, at completion of conjugation, fail to separate normally and remain united for variable lengths of time in the region of the paroral cone. In a cross as shown in Figure 3, the F1 sensitive conjugant of the pair remains sensitive if no cytoplasm is exchanged with the killer mate. If, however, cytoplasm from the killer conjugant gets into the sensitive mate, the latter will develop killing, the rapidity with which it becomes a killer being directly correlated with the amount of killer cytoplasm received. The results of this and other experiments cited above parallel the results described by Sonneborn (1943, 1945) for the way in which the original killer character is inherited. All other mutants thus far analyzed have yielded similar results.

These various mutant cultures are best described by the types of kappa they were found to contain. One contains a kappa comparable to the m2 kappa already described: it produces the spinner type of killing. Unlike the mutant in which m2 kappa was discovered, however,

this second mutant lacks m5 kappa. Whereas the presence of m5 kappa protected the animal from the lethal action of the original paramecin, this mutant, lacking m5 kappa, is now sensitive to the original killer. Consequently, it kills the original but, in turn, is killed by the original killer.

The third mutant may be called the "super killer." It contains a kappa identical with or similar to the m2 kappa, producing the spinner type of killing action. In addition, it contains original kappa and produces strong original type killing.

Finally, the fourth mutant has a kappa that acts very much like m5 kappa, but is intermediate between m5 and the original in the amount of detectible paramecin produced.

Two general types of kappa can thus be distinguished: one type includes three mutant kappas which are very similar to or identical with one another and differ from the original kappa in at least two respects, the type of paramecin produced and a slower rate of multiplication. The second general type includes two kappas strongly resembling the original kappa both in type of paramecin produced and in rapid rate of multiplication, differing only in the amount of paramecin detected. It is a curious fact that in at least two of the four spontaneous mutants, the two general types of mutant kappas have been found together in the same cell. It would seem that this apparent coincidence of two mutations must have special significance, but what it may be is not yet known. Further, the fact that we can find at least two kinds of kappa co-existing in the same cell and under control of the same gene opens a field for the study of competition between cytoplasmic factors, from which we might gain insight into how diverse changes in cell functions and phenotypes occur in the absence of genic change. The main result of this study, however, is the demonstration that kappa

can mutate, a property which it shares with genes and viruses.

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## THE KILLING SUBSTANCE, PARAMECIN: ACTIVITY OF SINGLE PARTICLES

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*Different types of paramecin.* Paramecin is the term used for the killing substance formed by any of the killer stocks of *Paramecium aurelia*, of which there are several in varieties 2 and 4. Since the paramecin produced by each killing stock has its own specific and characteristic effect on sensitive stocks, it is evident that actually there are a number of different paramecin-substances, though it seems likely that they are closely allied. To indicate which stock is responsible for the particular paramecin under discussion, the stock number or letter is used after the word paramecin.

*Location of paramecin in the body as well as in the fluid.* As early as 1939 Sonneborn discovered that paramecin is present in the fluid in which killers have lived and from which they have been removed, for sensitives placed in such fluid are killed with the same sequence of characteristic changes preceding death as occurs when they are placed directly with the killers.

In 1946, Sonneborn, Jacobson and Dippell demonstrated that paramecin is also present in the bodies of the killers, for they found that the killing power of fluid from a concentrated mass culture of stock 51 is increased by three or four times when the bodies of the killer animals in the fluid are broken up by repeatedly squirting them through a 25-gauge injection needle.

*Activity of single particles of paramecin.* Studies made during the last two years have produced evidence concerning the activity of single particles of paramecin and their rate of production. In my experiments, a definitely known small number of killers was used; while in a group of parallel studies on the same problem, Sonneborn, Dip-

pell and Jacobson (1947) have used large numbers of killers. In both sets of experiments the killer stock employed was Sonneborn's stock 51 of variety 4; and the sensitive stock was his stock 31 of variety 8. In both cases, the general procedure was used in two ways. In the first, the number of sensitive animals killed was determined *when the amount of paramycin-51 was varied* while the number of sensitive animals used was approximately the same. In the second, *the number of sensitives used was varied* while the amount of paramycin-51 was kept constant.

#### WHEN THE AMOUNT OF PARAMYCIN IS VARIED

1. *Results when known small numbers of killers are used.* The first method of varying the amount of paramycin, used in the experiments of Austin (1946, 1947), is to vary the number of killer animals that have lived in the fluid for a standard interval of time; for clearly the more killers there are, the more paramycin will be liberated into the fluid. Under the conditions employed, five hours represents the time that ordinarily elapses between two successive divisions; this five-hour interfission period was the standard time-interval adopted. The procedure used was as follows: Into 0.1 to 0.2 cc. of fluid 1, 2, 4, 8, 16 and 32 killers were introduced just after the completion of a fission. Five hours later, just before they had divided again, the killers were removed and 100 to 200 sensitives were added to each test. The number of dead or dying sensitives was determined by observations on the three following days. Forty series of tests of this kind were conducted.

The mean numbers of sensitives killed are shown in Fig. 1. The diagonal line represents the theoretical straight-line curve that would be expected if there were a perfect 1:1 relation between the number of killers and the number killed. The actual results obtained have been placed in relation to this theoretical curve. As the graph shows, the average number killed rises with an increase in

the number of killers. In the first four groups of tests (those with 1, 2, 4 and 8 killers) the numerical agreement between the average number killed and the number of killers is very close. In the earlier experiments when 16 and 32 killers were used, the number killed was considerably less than the number of killers. Later experiments showed that the explanation for these discrepancies lies in the fact that in the removal of the larger numbers of killers from a volume as small as 0.1 or 0.2 cc., some of the paramecin is carried away in the fluid. When the volume of fluid in which the killers were left for five hours was increased to 0.45 cc., the mean number killed by 32 killers

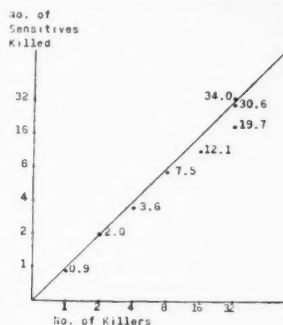


Figure 1.

was 34; or when precautions were taken to recover in a second volume of fluid paramecin picked up with the killers from the first, the mean number killed was 30.6. With such changes in technique, then, the numerical correspondence between the number of killers and the mean number killed is very nearly perfect even when the larger numbers of killers are employed.

2. *Results when a series of different dilutions of fluid containing large quantities of paramecin are used.* A second way of varying the paramecin content was employed by Sonneborn, Dippell and Jacobson (1947) by the method already referred to for getting heavy concen-

trates of paramecin. The data of these authors, not yet published in full, are given here with their permission. Mass cultures of killers were first concentrated by centrifuging; then the bodies of the animals were disintegrated by squirting them repeatedly through a fine-gauge injection needle. This material was diluted to different concentrations and the killing potency of 0.1 cc. samples of each concentration was determined by adding approximately 1000 sensitives to each test and noting the number killed in 48 hours. Figure 2 shows the results of a typical

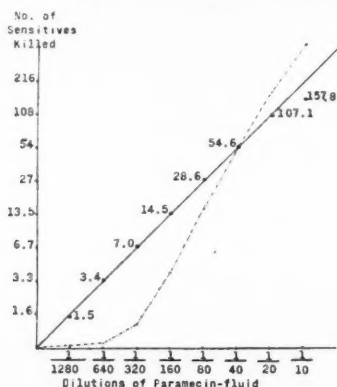


Figure 2.

experiment. The solid diagonal line gives the theoretical results which would be expected if the number of sensitives killed was exactly doubled each time the concentration of the paramecin-fluid was doubled, as would be the case if each particle of paramecin killed a different sensitive animal. A theoretical value of 54 (very close to the actual value of 54.6) was assumed for the 1/40th dilution, to serve as the base of calculations in plotting the theoretical curve. As is seen, the observed values lie very close to this theoretical curve. The broken curve gives the theoretical results, calculated by the use of the Poisson series, that are expected if a minimum of two particles of paramecin is required to kill one sensitive animal. Note how

greatly this curve diverges from the observations. If more than two particles were required to kill one animal, the divergence from the actual results would be even greater.

When the same type of analysis is applied to the results of the experiments shown in Fig. 1, on the assumption that two or more particles of paramecin are required to kill one sensitive animal, the theoretically expected numbers killed by 1, 2, 4, 8, 16 and 32 killers are 1, 2, 6, 19, 48 and 83. Both types of experiments thus lead to the same conclusion: each particle of paramecin can kill one sensitive animal.

#### WHEN THE NUMBER OF SENSITIVES IS VARIED

1. *Results of varying the number of sensitives at each constant level of paramecin in a series of small known numbers of killers.* The same conclusion is forced upon us by a second type of experiment, in which the amount of paramecin is kept constant, but the number of sensitives exposed to it is varied. In a series of four experiments the number of sensitive animals was varied from 120 to 2400 at each constant level of paramecin dosage in the series of 1, 2, 4, 8, 16 and 32 killers, in 0.2 cc. of fluid (Austin, 1946, 1947). Sample counts were made of a dense centrifuged culture of sensitives, then this dense culture was diluted to various concentrations with the supernatant fluid. Figure 3 shows graphically the results when four killers were used. No matter how many sensitives were employed between 120 and 2400, the mean number killed was approximately four. This is what would be expected on the single-particle hypothesis; but on the two-particle hypothesis, we should expect that the number killed would vary, as the broken curve in Fig. 3 shows, with a variation in the number of sensitives.

At other levels of paramecin dosage, the divergences between the observed results and the theoretical results on the basis of the two-particle hypothesis are so great

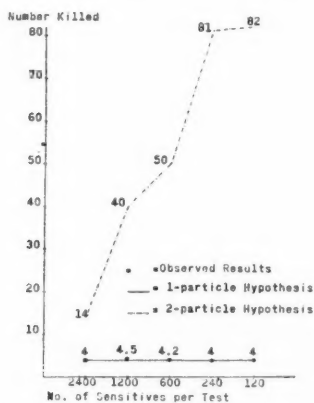


Figure 3.

that it is difficult to show the two sets on one graph. These have therefore been summarized in tabular form in Table I. In each horizontal row in this table, the upper figures, in the line opposite *a*, give the observed results; the lower

TABLE I.  
RESULTS OF VARYING THE NUMBER OF SENSITIVES  
Mean No. of Sensitives Killed

No. of Killers		No. of Sensitives per Test				
		2400	1200	600	240	120
1	a.	1	1	1	1	1
1	b.	1	2	3	9	14
2	a.	2	2	3	2	2
2	b.	4	7	14	29	40
4	a.	4	4.5	4.2	4	4
4	b.	14	40	50	81	82
8	a.	10	10.3	8.6	6.6	9.5
8	b.	56	100	148	166	115
16	a.	15	12.8	16.2	14.1	16
16	b.	200	298	342	230	120
32	a.	24	21.5	22	21.3	25
32	b.	596	685	437	239	120
Mean of means		a. 0.9	0.9	1.0	0.9	0.9
		b. 7.4	11.3	13.1	14.4	12.6

a: Observed Results

b: Theoretical Results on the 2-particle Hypothesis

ones, opposite *b*, give the theoretical results on the two-particle hypothesis. Here again it is evident that regardless of the number of sensitives used between 120 and 2400, the number killed at each level of killers closely approximates that number of killers in accordance with expectations on the one-particle hypothesis, while the theoretical results on the two-particle hypothesis diverge widely from this number.

2. *Results in varying the number of sensitives when the concentration of paramecin, obtained from the bodies of killers, is kept constant.* Sonneborn, Dippell and Jacobson (1947), using their method of releasing paramecin from the disintegrated bodies of killers, obtained similar results. For example, employing such a preparation of paramecin, and exposing 2000 to 62.5 sensitives to equal volumes of it, they found, as Fig. 4 shows, that the number killed remained essentially constant. The observed results are once more close to the theoretical expectations on the basis of the one-particle hypothesis and diverge widely from those on the basis of the two-particle hypothesis.

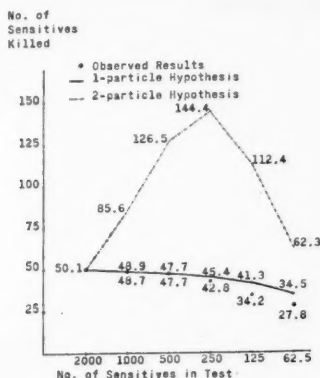


Figure 4.

*Rate of production of single particles of paramecin.* It has been mentioned above that in the experiments of Austin (1946, 1947) the approximate 1:1 relation between

the number of killers and the average number killed was found to hold for each interfission period of five hours. Table II shows the results of a series of experiments in which the killers were transferred to a new volume of fluid at the end of each hour for five hours, and sensitives were added to the fluid from which the killers had just been removed. As the table shows, the total number of sensitives killed still approximates the number of killers used, just as it does in the undivided five-hour period.

TABLE II.  
NUMBER OF SENSITIVES KILLED  
IN 5 SUCCESSIVE 1-HOUR PERIODS

No. of Killers	No. of Sensitives Killed in 5 1-hr. Periods					Total No. Killed in 5 Hrs.	Mean No. Killed per Killer
	I	II	III	IV	V		
70	20	22	16	8	10	76	1.08

In other experiments of this type, the five-hour period was subdivided into longer and shorter intervals than one hour; and varying numbers of killers were used. Experiments with single killers all showed what the one summarized in Table III does: That the killing effect of one killer in five hours is not diminished by such transfers to different fluids at the end of short intervals, but is manifested in full effect in one, and only one, of the fluids in such a series. This might be expected if paramecin is produced as single discrete units at the average rate of one per killer in each five-hour period.

TABLE III.  
RELATION OF KILLERS TO KILLED  
IN 9 SUCCESSIVE 35-MINUTE PERIODS

No. of 51VIII Killers	No. of 31VIII Sensitives Killed									Totals	
	Periods:	1	2	3	4	5	6	7	8	9	
1		1	-	-	-	-	-	-	-	-	1
1		-	-	-	1	-	-	-	-	-	1
1		-	1	-	-	-	-	-	-	-	1

*Conclusions.* The results of all these various types of experiments lead to the following conclusions:

- (1) Paramecin occurs in the form of unit-particles.
- (2) Under the conditions of my experiments, killers produce in the culture medium, on the average, one unit-particle of paramecin per killer animal per five hours.
- (3) A sensitive paramecium can be killed by a single unit-particle of paramecin.

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## THE KILLING SUBSTANCE PARAMECIN: CHEMICAL NATURE<sup>1</sup>

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From accumulated evidence it is known that both the cytoplasmic factor *kappa* and paramecin act in extremely minute quantities. Sonneborn (1946) has previously pointed out that the biochemistry of these factors might be of a sort very different from that usually visualized, in which the laws of mass action are supposed to operate.

Biochemical investigations of the nature of the gene *K-kappa*-paramecin system were initiated about a year ago. Since the presence of paramecin, the end product of this system, can be easily demonstrated and quantitatively determined, it seemed advantageous to attack the system in *Paramecium* from this end. No new techniques for testing the presence of this factor in solutions had to be developed and the work could proceed from the solid foundation laid by Sonneborn and associates. Once the properties and the chemical character of paramecin are established, one might predict with more certainty the character of its determinant *kappa*, and thereby facilitate the experimental approach to the whole system.

Sonneborn, Jacobson and Dippell (1946) had already presented evidence indicating the extreme instability and the apparent large molecular size of paramecin. Before any successful attempts towards isolation could be made, a further investigation of the properties of para-

<sup>1</sup> Contribution No. 382 from the Department of Zoology, Indiana University.

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mecin was deemed necessary. We have investigated the effect of the hydrogen ion concentration upon the stability of paramecin at different temperatures (van Wagtendonk and Zill, 1947) and the effect of several enzyme preparations (van Wagtendonk, 1948).

Experiments of this kind and the conclusions derived from them are only valid if there exists a proportionality between the amount of paramecin present in a solution and the number of animals killed by that solution. Dr. Austin has just presented you with the evidence that such a proportionality indeed exists, and we have confirmed these experiments repeatedly. Hence, the number of sensitive animals killed will be used as a measure of the activity of samples of paramecin.

Our paramecin preparations were prepared by concentrating killer animals of stock 51, variety 4. The concentrates were suspended in an appropriate phosphate buffer, and this suspension was forced through a narrow gauge needle. After approximately 10 passages through the needle usually all animals were broken up, though occasionally a few intact animals remained. These were removed. This suspension of cell debris and protoplasmic material, which will be referred to as the "brei," was used in all the experiments.

In the pH experiments the brei was adjusted to the desired pH and the test tubes containing the brei were placed in a constant temperature bath at 30° C. Immediately after temperature equilibration a sample was taken and subsequent samples were removed at 10 minute intervals. The samples were diluted 1:500 with phosphate buffer of pH 7.0 and were tested in the usual way for activity. A control at pH 7 was included in each experiment. In all the experiments the activity at zero time was arbitrarily taken as 100 per cent. The activity at the different time intervals was calculated as the percentage activity remaining at that time.

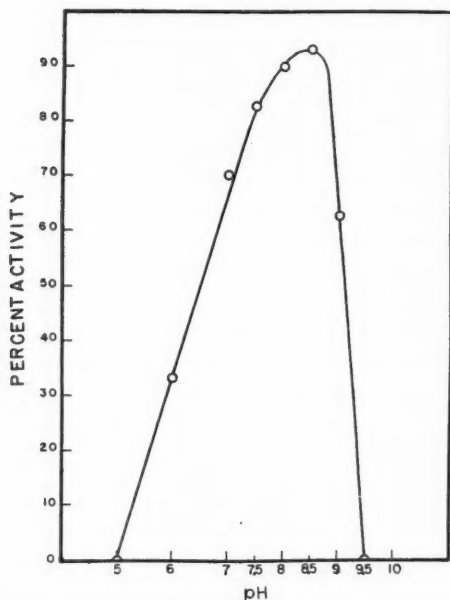


Figure 1.

In the Figure 1 the percentage activity left after one-half hour incubation is plotted against the pH. Paramycin has a narrow pH stability range in the alkaline region. The compound is very unstable, evidenced by the fact that even at the pH where it appears to be most stable, pH of 8.5, 7 per cent. of its initial activity is lost after one-half hour incubation. No activity is left at pH 5 or pH 9.5 after this time.

The relation between the stability of paramycin and the pH is clear from Figure 2. The percentage activity left at each time interval is plotted against time on a semi-logarithmic scale. The slope of the curve at pH 6 is the steepest, only 13 per cent of the activity remaining after one hour. Fifty per cent. of the activity is left at pH 7,

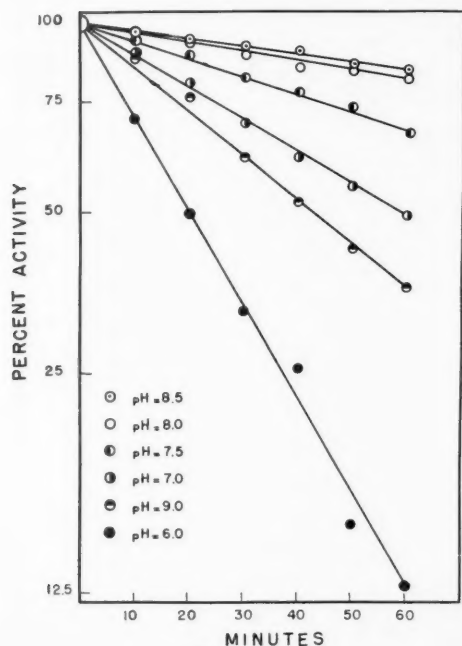


Figure 2.

68 per cent. at pH 7.5, 82 per cent. at pH 8.5, and 39 per cent. at pH 9. The points fall along a straight line, indicating that the inactivation of paramecin is a mono-molecular reaction. Inactivation proceeds much more rapidly at 40° c. Only 10 per cent. of the activity was left after a one hour incubation at 40° at pH 7 as compared to 50 per cent at the same pH at 30°. The heat of activation of the inactivation reaction of paramecin could be calculated. This value is for pH 7, 126,000 cal. per mole, a value which is typical for enzymes and proteins. It can be concluded from these experiments that paramecin belongs to either of these two classes of compounds or to both.

The analysis of paramecin by means of enzyme digestion experiments which seek to identify specific sub-

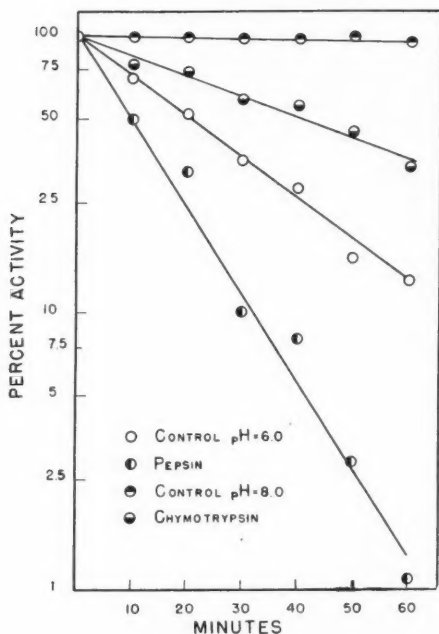


Figure 3.

stances and linkages by specific enzymes might reveal some of the chemical nature of this compound. Methods of this kind have been invaluable in the analysis of the chemical nature of the chromosome by van Herwerden (1913), Caspersson (1936), Mazia (1941) and Catchside (1947). The method has been criticized on several occasions, especially since the criteria for enzyme purity are not a guarantee against contamination by other enzymes, most of the enzymes being of protein nature. I will discuss here only those enzyme experiments which gave direct information on the structure of parmeicin.

A brei was incubated with pepsin at a pH where this enzyme still shows proteolytic activity. The inactivation proceeded much more rapidly than the inactivation in the

control at the same pH. Only one per cent. of the activity was present after a one hour incubation as compared to 13 per cent. in the control. The inactivation of paramecin in the presence of chymotrypsin proceeded also more rapidly than in the corresponding control at pH 8, 30 per cent. of the activity remaining after a one hour incubation as compared to 83 per cent. in the control. This indicates that a protein structure is essential for the activity of paramecin.

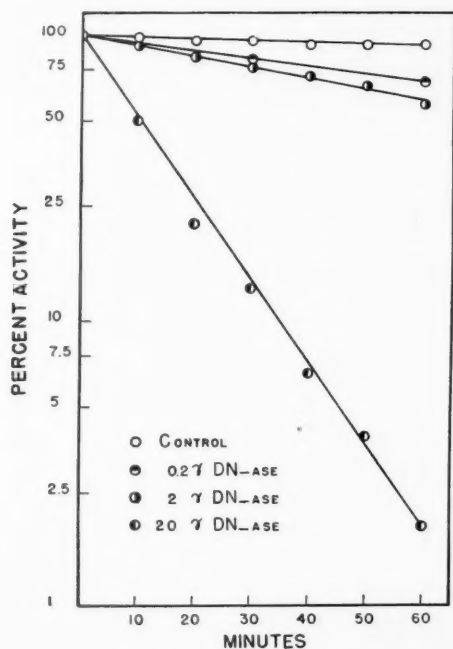


Figure 4

The most revealing experiments were those carried out with desoxyribonuclease. Paramecin was incubated in the presence of magnesium and gelatin with desoxyribonuclease in concentrations of 20 γ, 2 γ, and 0.2 γ. Mg was added to insure the highest activity of the desoxyribonu-

clease, while gelatin was added to prevent denaturation of this enzyme. Inactivation was rapid in the highest concentration of desoxyribonuclease and proportionally slower in the lower concentrations. That this is not due to the presence of Mg in the substrate is clear from the fact that the control containing both Mg and gelatin showed only a slight inactivation in the investigated time interval.

There has been some doubt as to the specificity of the desoxyribonuclease preparations, especially since Laszkowski (1947) had been able to isolate a specific proteinase from desoxyribonuclease preparations. In order to prove that the observed inactivation of paramecin was due to the action of desoxyribonuclease, several specific activators and inhibitors were investigated. Magnesium specifically activates desoxyribonuclease in concentrations as low as 0.003 M. The action of magnesium is counteracted by citrate.

TABLE I. INFLUENCE OF  $Mg^{++}$  AND CITRATE UPON THE INACTIVATION OF PARAMECIN BY DESOXYRIBONUCLEASE

	Per cent Activity			
	0 min.	20 min.	40 min.	60 min.
brei	100.0	93.7	91.7	90.0
brei + Mg	100.0	96.7	94.2	91.0
brei + citrate	100.0	92.5	92.0	88.8
brei + DN-ase	100.0	96.0	92.0	91.0
brei + DN-ase + Mg	100.0	23.4	6.7	3.3
brei + DN-ase + Mg + citrate	100.0	94.8	91.8	87.5

In the first experiment of Table I, serving as an overall control, the brei was incubated without any additions. In the second experiment magnesium ions were added to the substrate; in the third, citrate; and in the fourth, desoxyribonuclease. The inactivation in these three experiments was not essentially different from the one found in the control, approximately 10 per cent. in all cases. However, addition of both Mg and desoxyribonuclease to the brei resulted in a rapid inactivation of 97 per cent. of paramecin. When citrate was added, as in the last experiment, the inactivation of paramecin was not different

from that of the control, indicating inhibition of the desoxyribonuclease action, and thereby preventing the destruction of paramecin.

Manganese also acts as a specific activator of desoxyribonuclease. However, this activation is not inhibited by citrate ions. The inactivation in the brei plus manganese, the brei plus citrate, and the brei plus desoxyribonuclease was the same as that found in the brei alone, 10 per cent. However, when manganese and desoxyribonuclease were present together in the substrate, inactivation of paramecin was rapid. The addition of citrate did not change the rate of inactivation. Only about 3 per cent. of the initial amount of paramecin was found after one hour inactivation in both cases. (Table II).

TABLE II. INFLUENCE OF  $MN^{++}$  AND CITRATE UPON THE INACTIVATION OF PARAMECIN BY DESOXYRIBONUCLEASE

	Per cent Activity			
	0 min.	20 min.	40 min.	60 min.
brei	100.0	97.5	91.0	91.0
brei + Mn	100.0	95.6	93.7	91.7
brei + citrate	100.0	94.5	92.5	90.8
brei + DN-ase	100.0	94.2	94.2	92.0
brei + DN-ase + Mn	100.0	31.0	5.7	3.2
brei + DN-ase + Mn + citrate	100.0	31.6	6.1	2.5

The proteolytic enzyme, Protein B, presumably contaminating desoxyribonuclease, did not inactivate paramecin in concentrations 1000 times greater than the concentration of desoxyribonuclease. Eight per cent. of the initial paramecin activity was lost after a one hour incubation in the control, 9 per cent. when protein B was added in a concentration of 20 mg per cc and similar amounts in the 10 and 1 mg concentrations of this proteolytic enzyme. If it is taken into account that this proteolytic enzyme was present in only very small concentrations in the desoxyribonuclease preparations, it becomes at once obvious that the observed inactivation by desoxyribonuclease could never have been due to the contaminating proteolytic enzyme. (Table III).

TABLE III. THE ACTION OF PROTEIN B UPON THE ACTIVITY OF PARAMECIN

	Per cent Activity			
	0 min.	20 min.	40 min.	60 min.
brei, pH equals 7.8	100.0	98.0	92.0	92.0
Protein B, 20 mg. per cc.	100.0	97.2	94.5	91.0
Protein B, mg. per cc.	100.0	97.2	94.5	91.0
Protein B, 1 mg. per cc.	100.0	90.7	87.5	85.5

From these experiments it may then be concluded that paramecin is a desoxyribonucleoprotein, although it is fully realized that final and definite proof depends upon a successful isolation in the pure and active state.

If the isolation would give affirmative proof, it would imply a very close chemical relationship between the cytoplasmic factor kappa and its product, paramecin.

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# ANTIGENIC CHARACTERS IN *PARAMECIUM AURELIA* (VARIETY 4): DETERMINATION, INHERITANCE AND INDUCED MUTATIONS

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This paper deals with three subjects. Part I sets forth the mode of determination and inheritance of a group of similar traits that occur normally in *Paramecium*. Part II shows how subjection to a specific reagent brings about permanent hereditary changes in traits of this group. Part III is a study of the differences among the four different hereditary types and is designed to throw light on the nature of the experimentally induced changes reported in Part II.

## I

Table I shows how the traits are identified. Four diverse strains (A, B, C, and D) that arose within the race 51 of *Paramecium aurelia* (variety 4) were injected into different rabbits. Later, serum was obtained from each rabbit, diluted with 400 parts water, and tested as to its action by placing some paramecia in a drop of it. Each serum quickly paralyzes animals of the strain injected





ANTIGENICALLY DIVERSE STRAINS	 STRAIN A	 STRAIN B	 STRAIN C	 STRAIN D
	ANTI-A SERUM	ANTI-B SERUM	ANTI-C SERUM	ANTI-D SERUM
A	+	—	—	—
B	—	+	—	—
C	—	—	+	—
D	—	—	—	+

Table I. The four antigenically diverse strains and their reactions to anti-sera. All sera inactivated at 56 degrees C for 30 minutes and diluted with 400 volumes of water. — equals no effect on paramecia; plus equals paralysis of paramecia.

<sup>1</sup> Aided by grants from the Rockefeller Foundation and Indiana University.

into the rabbit from which it was obtained, but has no effect on animals of the other three strains. Each of the four strains (and any other culture like it) is thus identifiable by the serum which paralyzes it. The specificity of the paralysis reaction indicates that it is due to interaction between substances called antigens in the paramecia and specific complementary substances called antibodies which are formed in the rabbit in response to their presence. The four strains A, B, C and D are thus antigenically different.

Analysis of the genetic system involved in the control of these antigenic traits follows the pattern used for the killer trait (Sonneborn, 1943) and depends on the fact that when two paramecia conjugate, their nuclei come to have identical genes, but their cytoplasms remain distinct. Hence the two mates of a conjugant pair must yield identical cultures for traits determined by genes, but diverse cultures for traits determined by cytoplasm.

The latter is the result obtained when conjugation occurs between antigenic types A and B: the A mate produces an A culture, the B mate a B culture. Inheritance thus seems to be cytoplasmically determined.

This is confirmed by the results when one examines conjugant pairs which become abnormally united so that cytoplasm visibly flows from one to the other before they separate. If the trait is indeed cytoplasmically determined, the two mates of such a pair might be expected to yield similar cultures. They do: both are type A or both type B, suggesting the existence of two cytoplasmic determiners, one for A and one for B.

When smaller amounts of cytoplasm are exchanged during mating, one mate produces a culture of type A while the other produces a culture in which most lines of descent are type B and some lines of descent are type A: or the reverse occurs. As all the animals are of the same genotype, the origin of two antigenically diverse lines of

descent from a single conjugant is further evidence that cytoplasm is here determinative.

Conversely, other evidence shows that these antigenic types do not differ in genes. If types A and B differed in genes, the genes would be expected to segregate in F<sub>2</sub>, but this does not happen. When either of the F<sub>1</sub> types is inbred, all the F<sub>2</sub> are like the F<sub>1</sub> parent. The antigenic types A and B thus differ only in cytoplasmic, not in genic, constitution. Similar results have been obtained for the antigenic type D. As antigenic traits are normal and universal in *Paramecium*, it would seem that whatever genetic system controls them must also be normal. It may therefore be concluded that the cytoplasmic factors or plasmagenes (which may be called the alpha series) determining these antigenic traits are normal and integral parts of the genetic system.

## II

These antigenic traits can be permanently altered by exposing animals to their specific type of antibody. In most of the work, for purposes of uniformity, the source of antibody was a mixture of the undiluted antisera, containing antibodies against all four types. This was then adsorbed to free it of all but one kind of antibody by adding animals of one type until they ceased to be paralyzed, then centrifuging these out and adsorbing successively in the same way with two other types, leaving antibodies against only the fourth type. The four adsorbed sera obtained in this way are thus specific reagents, each one paralyzing only one of the four types, as shown in Table I for the four sera before mixture and adsorption.

In work of this sort, the chief source of misinterpretation is the possibility that subjection to antibody merely selects for survival mutations that would have arisen anyway independently of exposure to specific antibody. This is currently the favored interpretation of work of this sort and has been adopted by Harrison and Fowler

(1945) in their antigen studies on *P. aurelia*. Hence, we must first determine the frequency with which such mutations occur under conditions comparable to those used in the experiments. For this purpose, exposures were made to agents that do not paralyze the animals: water, pooled antisera from which *all four* kinds of antibodies had been adsorbed out, and non-homologous antibodies, *i.e.*, antisera containing only antibodies against *other* antigenic types. In every case, the exposed animals were washed, isolated and allowed to produce a culture which was then tested with the various sera to discover, by means of the paralysis reaction, the antigenic type to which it belonged. All of the non-paralyzing agents gave essentially the same results, so the totals for all are presented together in Table II. The total for type A was 4 changed cultures out of 3809 examined or 0.11%; type B gave 0.06% and type D, 0.53% changed cultures. Mutations of types A, B and D are thus rare events. Type C, on the other hand, proved too unstable for work of this sort and will be discussed no further.

Table II. Frequency of origin of antigenically changed cultures produced from single animals. Controls exposed to water and to adsorbed sera *lacking* paralyzing antibodies against them. Experimentals exposed to sera *containing* paralyzing antibodies against them.

Antigenic Type		A	B	D
Controls	No. Exposed	3809	3474	2636
	No. Changed	4	2	14
	% Changed	0.11	0.06	0.53
Experimentals	% Changed	22.22	47.40	90.40
	No. Changed	40	439	706
	No. Exposed	180	926	781

Even though types A, B and D mutate with low frequency, one must still preclude the selection of these rare mutations in the experimental groups exposed to specific paralyzing antibodies. In these exposures, *all* the paramecia were paralyzed, proving that all were of one type at the start and excluding the presence of mutants in the exposed material. Further, the numbers of paramecia

exposed per unit volume, the concentration of antibody, and the exposure time were so adjusted that practically no paramecia were killed, none reproduced and practically all recovered from the paralysis, thus making appreciable selection of spontaneous mutants impossible.

Yet changed cultures arose in greatly increased frequency, as shown in Table II. Type A yielded 22.22 per cent; type B, 47.40 per cent and type D, 90.40 per cent changed cultures. The frequency of change has been increased 200 to 800 times. These results, based on studies of nearly 17,000 exposed animals and the progeny obtained separately from each of them, show beyond question that exposure to specific paralyzing antibody induces change of antigenic type.

Table III. Percentage of antigenically changed cultures obtained from groups of 120 isolated animals of antigenic type D that had been exposed to anti-D serum.

Exposure (Minutes)	Concentration of Antiserum		
	.001	.01	.02
12	0	67	80
120	0	95	98
1200	0	100	98

Table III presents results that confirm this conclusion quantitatively. It gives the percentages of changed cultures obtained from groups of 120 animals of type D individually cultured after exposure to various concentrations of antibody against type D and for various periods of time. The percentage rises from 0 to 100 per cent as exposure time and the concentration of antibody are increased. Similar results were obtained with the other antigenic types.

Practically all of the 1200 animals that produced altered cultures changed from one to another of the four types A, B, C and D. Few belonged to previously unknown antigenic types. Moreover, when a change of type occurred, the new type remained as constant as the original strain of that type. Some have been under observa-

tion for over five months, during which hundreds of cell-generations and more than a dozen successive fertilizations have taken place. These, like those not yet followed so long, maintain their new antigenic type. In sum, the changes are hereditary; apparently they are mutations. Since they were experimentally induced by exposure to specific antibody only, they seem to be directed mutations; but they are directed mutations of *cytoplasmic factors*, not of genes, as the breeding experiments presented earlier show.

In respect to their permanence, they seem to differ markedly from the changes of antigenic type induced in similar ways earlier by Jollos (1921), by Sonneborn (1943) and by Kimball (1947), for the changes they observed persisted only for a limited period of reproduction or invariably reverted to the original type at the first fertilization. Most, and possibly all, of the earlier work was done on strains in which genes seemed to determine the traits. Apparently, when the antigenic traits are cytoplasmically determined, as in our present material, antibody induces *permanent* changes.

### III

An attempt has been made, by immunological methods, to obtain insight into the nature of the cytoplasmic differences among the four antigenic types and hence into the nature of the induced mutations. Table I shows that each antigenic type is paralyzed only by its own antiserum when the antisera are diluted 1:400. A very different picture is obtained when one prepares a series of dilutions and determines the lowest concentration that will paralyze the animals. As shown in Table IV, each antiserum can paralyze not merely one, but three or four, of the types. However, to paralyze non-homologous types, the antisera must be used in much lower dilutions.

Table IV. Lowest paralyzing concentration of antisera. Concentration expressed as ratio of volume of antiserum to volume of diluent (water).

Antigenic Type of Paramecia	Antiserum			
	A	B	C	D
A	1:800	1:50	1:100	1:1000
B	1:25	1:3200	1:200	1:200
C	1:3	1:6	1:1600	
D	1:12.5		1:3	1:3200

The meaning of this is discoverable by adsorption studies, as shown in Table V. When anti-C serum is adsorbed with type A animals until it can no longer paralyze them, it can still paralyze the other types in about the same dilutions as before adsorption. Hence, the antibodies in the serum that paralyze type A are not the same as the ones that paralyze the others. In like manner, adsorption with type B takes out the antibodies that paralyze it, but leaves the others essentially untouched; and similarly for type C.

Table V. Lowest paralyzing concentration of anti-C serum.

Antigenic Type of Paramecia	Anti-C Serum			
	Unadsorbed	Adsorbed by Antigenic Type		
		A	B	C
A	1:100		1:100	1:100
B	1:200	1:200		1:200
C	1:1600	1:800	1:800	

Anti-C serum thus contains three kinds of antibodies. Further, the three kinds of antigens on which they act must all have been in type C animals, for the rabbit produced all three kinds of antibodies in response to the injection of type C animals. Moreover, of these antigens in type C, one is indistinguishable from the paralysis antigen in type A, for antibodies against type C also paralyze type A. In like manner, it may be shown that one of the antigens in type C is indistinguishable from the paralysis antigen in type B. However, the antibodies that paralyze types A and B are present in C serum in relatively small amounts; this suggests that the antigens involved in the paralysis reaction of types A and B are

present in relatively small amounts in type C animals. On the other hand, the antigen in type C which is involved in its paralysis is, by the same reasoning, probably present in much larger amounts, for the antibody against it is the main one in the anti-C serum. This predominant and paralyzing antigen in type C animals may be called its primary antigen, the others its secondary antigens.

Table VI. Antigenic constitution of the four antigenic types. The antigen involved in the paralysis reaction is called the primary antigen; it is the one present in largest amount. The other antigens are called secondary antigens.

Type	A	B	C	D
Primary Antigen	1	2	3	4
Secondary Antigens	2,3,4	1,3	1,2,4	1,2

Similar analyses have been carried out for the other antisera and similar results have been obtained. These indicate that the antigenic constitution of the four types is as shown in Table VI, in which the primary antigen of each type is represented by the numbers 1 to 4. Animals of each antigenic type have a relatively large amount of their primary antigen which functions in the paralysis reaction, and relatively little of the two or three possible secondary antigens. Thus all four of the antigenic types seem to be similar, if not identical, in the kinds of antigens they contain and differ chiefly in which antigen is the primary and in the relative proportions in which the antigens are present.

#### DISCUSSION, CONCLUSIONS AND SUMMARY

If, as seems likely, the antigens (1, 2, 3 and 4) are maintained and multiplied in all four antigenic types (A, B, C and D), there is available no evidence as to the basis of the determination and inheritance of the antigens, for no differences in this respect are involved in the crosses and breeding analysis. The only difference that can be analyzed in this way is the difference with respect to which of the four antigens is primary (antigen 1 in type A, antigen 2 in type B, and so on), for this is

the main and possibly only distinction among the four antigenic types. The primary antigen is the one present in largest proportion and it is also the only one participating in the paralysis reaction to antiserum. The breeding analysis shows that the antigenic types are genically alike and differ only in some cytoplasmic feature; that is, the determination as to which antigen shall be primary is cytoplasmic. Change of primary status from one antigen to another, *i.e.*, change from one antigenic type to another, may occur under either of two conditions: *first*, by acquiring much cytoplasm from another antigenic type; *second*, by subjection to specific antibodies against the original primary antigen. Both of these conditions probably change the proportions of the antigens: the first, by adding relatively large amounts of another antigen; the second, by inactivating a large proportion of the original primary antigen. These relations suggest that the four antigens can be maintained in only one of a small number of stable proportions and that any conditions which greatly disturb the existing stable proportion may lead to the establishment of a different stable proportion. The mechanism by which the stable proportions are attained and maintained is unknown, but it is not unlikely that the antigens themselves are autosynthetic, *i.e.*, they may themselves be plasmagenes. At least it seems at present necessary to conclude that the maintenance of an antigen as primary is cytoplasmically controlled. In this sense, cytoplasmic factors or plasmagenes play the decisive role in determining and maintaining the antigenic types.

The hereditary transformation of one antigenic type into another by means of exposure to specific antibodies against the primary antigen seems to be an example of directed mutation of a particular trait by a specific reagent active on that trait only. Although these changes are mutations in the broad sense of permanent changes

in hereditary traits, they do not involve changes in the kinds of antigens present and therefore objections might be raised to referring to them as mutations. However, the changes seem to be more than mere changes in the proportions of pre-existing antigens, for the antigen previously involved in the paralysis reaction ceases to operate in this way and one which previously did not, now does. Regardless of whether the term "mutation" is appropriate for such an hereditary change, the phenomena may present a clue for the understanding of how cells of the body of higher organisms become persistently diverse (even during long growth in tissue cultures) in spite of their having the same kinds of genetic determiners. The persistently diverse cell-types in the body, like the diverse antigenic types of *P. aurelia*, may have the same kinds of materials present in different stable proportions and these proportional differences may lead to different phenotypes not only *per se*, but also by bringing about correlated changes in traits comparable to those involved in the shift from one primary antigen to another.

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# THE ROLE OF CYTOPLASM IN INHERITANCE IN VARIETY 1 OF *PARAMECIUM AURELIA*<sup>1</sup>

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So far two sexually isolated varieties (2 and 4) of *Paramecium aurelia* have been considered in this symposium. In these varieties, the cytoplasm plays an important role in inheritance at conjugation. In variety 1 with which the present paper deals, it does not appear to do so. The differences between the varieties are brought out in Figure 1. Crossing two different stocks of variety

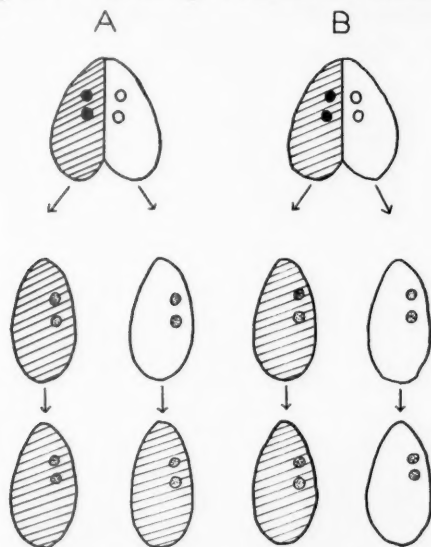


Figure 1. Diagram to show the differences between the A (variety 1 and others) and B (varieties 2, 4, and others) groups of varieties in inheritance at conjugation. Only the micronuclei, not the macronuclei, are shown. In both groups, the micronuclei of the two clones derived from a pair become identical. In the A group, the phenotype of the two members becomes identical while in the B group, differences which existed prior to conjugation persist.

<sup>1</sup> This investigation was aided by a grant from the American Cancer Society.

<sup>2</sup> Present location: Biology Division, Oak Ridge National Laboratories, Oak Ridge, Tenn.

1 produces identical clones from the two members of a pair in accordance with their identical genic constitutions. Crossing two different stocks in variety 4 produces clones which retain the characters of the stocks from which their cytoplasm is derived, despite the fact that they can be shown to have identical genes. The facts are reviewed by Sonneborn (1947).

Although cytoplasmic inheritance cannot be demonstrated at conjugation in variety 1, there are some phenomena of inheritance during vegetative reproduction which appear to be based upon changes in the cytoplasm. Jollos (1921) produced such changes, called by him *Dauermodifikationen*, by various treatments and showed that they could persist for many fissions before finally disappearing. We do not know, of course, with which, if any, of the now recognized varieties he was working. However, Sonneborn (1943) has reported a *Dauermodification-like* change in reaction to homologous antiserum in stock 60 of variety 1.

Phenomena which bear some similarity to *Dauermodifikationen* are known in varieties 2 and 4. The previous papers in this symposium show how changes in the killer character can be produced by conditions which reduce the quantity of kappa. Such changes persist when the animals are kept under conditions which do not favor the increase of kappa but eventually may disappear if kappa is not completely removed. However, and this is the major difference between those changes which have been described as *Dauermodifikationen* and the killer character, if kappa is completely removed the animals do not revert to killer. The question then arises how far the evidence warrants a uniform interpretation for the phenomena of cytoplasmic inheritance in varieties 2 and 4 and *Dauermodifikationen* in variety 1.

I have recently (1947) published some evidence on a *Dauermodification-like* change in reaction to homolo-

gous antiserum in stock 60 of variety 1 which I believe throws some light on this matter. The facts are these: Homologous antiserum in a standard concentration regularly immobilized and killed all animals of this stock. Spontaneous resistance to the antiserum was found only once in many hundreds of tests involving thousands of animals. However when animals were exposed to sublethal concentrations of antiserum or to the enzyme trypsin for twenty-four hours, a high percentage of the survivors was no longer immobilized or killed by the standard concentration of antiserum. This was true even after treatment with antiserum so dilute that none of the animals were killed, and true under conditions in which selection of spontaneously occurring resistant animals could be excluded as a factor.

Resistance could also be produced by culture at 14° C instead of the usual 27-29° C. In this case, all lines kept in isolation at this temperature sooner or later became resistant, the number of fissions required varying from one to more than one hundred.

Resistance induced in any one of these three ways persisted in isolation culture after removal from the inducing conditions for varying numbers of fissions as shown in Table 1. Different lines of the same clone varied in this respect as much as did different clones.

TABLE I  
Inheritance of Resistance Following Removal from the Inducing Conditions

Duration of Resistance in Number of Fissions	Number of Lines Showing a Given Duration Method of Induction		
	Antiserum	Trypsin	Cold
1-5	30	2	36
6-10	19	6	8
11-15	13	9	5
more than 15	9	18	33

It is clear that these changes could not be the result of gene mutation. The high percentage produced, taken in conjunction with the immediate appearance after treatment and the fact that induction took place during vege-

tative reproduction in an organism with diploid micronuclei and a compound macronucleus, is sufficient to virtually rule out this interpretation.

It is also clear that the transmission could not be the result of a simple carry-over of modified cytoplasm. Cytoplasm modified at the time of treatment would be rapidly diluted by new cytoplasm formed over the course of a few fissions. Thus after five fissions only about  $1/32$  of the cytoplasm of any one animal could be derived directly by carry-over of old cytoplasm from its progenitor five fissions back. Thus we are left with the conclusion that it is the cytoplasm which is modified by the treatment but that this modification must in some fashion affect the new cytoplasm which is formed after the treatment has ceased.

What is the nature of the original modification of the cytoplasm and how does it affect the new cytoplasm formed after treatment? The fact that resistance can be produced either by exposure to antiserum or to trypsin suggests that the first step may be the destruction or inactivation of antigens. If this were so, one interpretation of the long retention of resistance after treatment would be that the antigens are self-reproducing entities or at least that they facilitate the production of more of themselves. Then partial removal or inactivation would be expected to decrease the amount formed in subsequent fission intervals. The effect of low temperature could be attributed to a reduction in the rate of formation relative to the fission rate of the animals. This is essentially the interpretation which was presented as most probable in my recent paper. The interpretation is obviously similar to that for the killer case in varieties 2 and 4. However, one important difference exists. In the case of killer, permanent non-killer lines can be produced as a result of the complete loss of kappa. Attempts to produce lines permanently resistant to antiserum in stock 60 were not successful. This failure is in line with the differences

already known for inheritance at conjugation in the two groups. Sonneborn (1947) has suggested as one possible interpretation of the differences at conjugation that the genes in variety 1 are capable of initiating the production of cytoplasmic factors whereas those in variety 4 are not. If such a situation existed, then lines permanently resistant to antiserum would not be expected in variety 1 even after complete removal of antigens since the genes would be capable of starting their production anew.

Sonneborn's studies of antigenic changes in variety 4 which were presented in the preceding paper have led him to a rather different interpretation. The evidence in his case points to sudden changes in the relative proportions of several antigens as the basis for the change in specificity. Moreover, there seem to be only a few stable proportions possible. My results could also be interpreted in this way for there is no evidence which clearly shows that return to sensitivity is a gradual process. The return could occur suddenly but at different times in different lines of descent. As a matter of fact, a better explanation might be given in this fashion for the great variation in time required for reversion.

There still remain differences between the varieties. In variety 1, there appears to be only one stable antigenic state for a given condition of culture. Other states may persist for a time but sooner or later change to the stable one. On the other hand, in variety 4 several different antigenic states seem to be more or less equally stable.

Whichever interpretation, gradual change in amount of antigen or sudden shifts in the relative proportions of several, is *put upon* the results, it is still true that it would be very difficult if not impossible to interpret them without the aid of some mechanism for self-reproduction of cytoplasmic materials. Thus it seems probable that such self-reproducing cytoplasmic materials occur generally in *Paramecium aurelia* and are not just a peculiar feature of certain varieties. The difference between the

varieties in inheritance at conjugation and the correlated difference in inheritance of changes produced within clones still remains to be explained, but they are certainly not evidence that self-reproducing cytoplasmic entities are confined to a few varieties.

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## THE NATURE AND MODE OF ACTION OF THE MATING TYPE SUBSTANCES<sup>1</sup>

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Mating type inheritance in group B, *Paramecium aurelia*, is explained in part by analogy with the gene-kappa-paramycin system. Group B (Sonneborn and Dippell, 1946) animals usually do not change mating type at conjugation. Thus one variety 4 conjugant, produces a type VII clone; its mate produces a type VIII clone. Occasionally, however, both conjugants produce type VII or type VIII clones, indicating in each case that one conjugant changed mating type. Similarly, change of type from VII to VIII or VIII to VII occurs at autogamy in many stocks. Sonneborn (1947) correlates change of mating type at conjugation with delay in separation of the conjugating animals. This delay in separation is associated with exchange of cytoplasm. Cytoplasmic factors for mating type included in the exchanged cytoplasm are believed to effect the change of type. Nothing further is known concerning mating type inheritance in group B. Since no mating type genes have been reported, it is at present necessary to regard cytoplasmic factors alone as the mating type determining agents. This scheme requires at least two cytoplasmic factors in each variety, one for each mating type. Furthermore, when applied to change of type at conjugation or autogamy, the scheme implies competition of these cytoplasmic factors for "effective substrate."

It is apparent from this brief discussion that assumptions concerning fundamental issues arise at once when

<sup>1</sup> The studies of Metz and Foley, which form a major part of this report, were aided by a grant from the National Institute of Health, U. S. Public Health Service.

one approaches mating type inheritance. Thus further investigation of mating types and the factors controlling them should add much to our knowledge of the relation between genes, cytoplasmic factors and characters. Although the studies to be reported here concern the physiology of conjugation, it is hoped that the methods, facts and points of view developing from this work on variety 4, *P. aurelia*, may eventually help to unravel the relationship between the mating type characters and the agents controlling them.

#### THE MATING REACTION AND CONJUGATION

Conjugation in *Paramecium* involves several types of union between the conjugants and a variety of internal changes in these animals. The initial step in conjugation is a superficial contact and adhesion of animals (Sonneborn, 1937). Under appropriate conditions this may take the form of mass agglutination and is referred to as the mating reaction. In fact, occurrence of the mating reaction on mixture of clones from diverse sources serves to distinguish and identify complementary mating types.

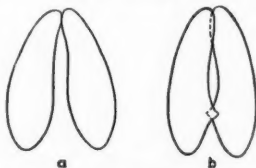


Fig. 1. Types of Union in conjugating, and pseudo-selfing paramecia.  
 a Holdfast union in early conjugants and pseudo-selfing animals.  
 b Holdfast and paroral cone union in more advanced conjugants.

Following the initial agglutination, potential conjugants unite in a more intimate but not inseparable fashion at a region near their anterior ends (Figure 1a) known as the *holdfast region* (Metz, 1947). Finally, the *paroral cones* (Diller, 1936), which have formed in the posterior region, unite (Figure 1b). From this time on the conjugants can not be separated.

Subsequent to holdfast union, but prior to paroral cone fusion, the conjugants lose their ability to give the ag-

glutinative mating reaction. This loss of mating activity results in breakdown of the mating reaction agglutinates into conjugating pairs. In *P. aurelia* the first signs of nuclear activity appear at about this time (Diller, 1936; Metz, 1947). These involve enlargement of the micronuclei and formation of the first meiotic prophase. Macronuclear breakdown or fragmentation begins shortly after completion of the second meiotic division. Nuclear behavior subsequent to meiosis will not enter into the discussion to follow.

#### PHYSICAL BASIS OF THE MATING TYPE DIFFERENCES

In any consideration of conjugation, and particularly in any analysis of the initial agglutinative mating reaction, it is essential to realize that hormones, gamones or sex stuffs are never found in the fluid from Paramecium cultures. Thus serious analogy with *Clamydomonas* (Moewus, 1939) or *Euplotes* (Kimball, 1943) is of little value. Consequently, consideration must be given at once to the surface structure of the Paramecium for an understanding of the mating reaction. More particularly, attention must be directed to the structure of the cilia, since Jennings (1939) has shown that the mating reaction involves these organelles. It seems reasonable to suppose that the agglutinative mating reaction results from interaction of definite complementary mating type substances or at least complementary configurations. This view is supported by several lines of evidence. Thus Sonneborn (1937, 1942) has shown that two paramecia of the same mating type can clump together temporarily if one of them has previously been in contact with an animal of opposite mating type. This indicates transfer of mating type substance from one animal to another. Boell and Woodruff (1941) and Metz (1947) have found that dead paramecia of one mating type will clump strongly and specifically with living animals of opposite mating type, again suggesting interaction of moderately stable sub-

stances. Furthermore, Metz (unpublished) has found that treatment of dead animals with antiserum inhibits the mating reaction between dead and living animals. Although this action of antiserum was not mating type specific, the results nevertheless indicate blocking of surface groups.

Direct examination of cilia has so far given no clue to the nature of the reactive surfaces. Thus Jakus and Hall (1946) observed bundles of fibrils in cilia by use of the electron microscope, but they were unable to determine what held these fibrils together or to detect a limiting sheath.

All attempts to extract mating substances from *P. aurelia* have so far failed (Metz, 1946a). Heating, grinding, freeze-thawing or extraction with acid, alkali, salt solution, urea, or organic solvents produced no fraction containing mating substance activity (specific action on animals of opposite mating type; inhibition of the mating reaction). Although large fragments of dead *P. aurelia* clumped with living animals, just as do fragments of living *P. bursaria* (Tartar and Chen, 1941), all mating activity disappeared when the animals were completely broken up by either physical or chemical means.

In a study of the effect of various agents on the mating type substances, Metz (1946a) found that *P. aurelia* could be killed by appropriate treatment with a rather wide variety of physical and chemical agents without destruction of mating activity. Such dead animals clumped strongly and specifically with living animals of opposite type. Unfortunately these results give little indication of the nature of the mating type substances. However, pursuit of this line of investigation should prove fruitful.

#### INDUCTION OF AUTOGAMY AND PSEUDO-SELFING PAIR FORMATION IN *P. aurelia*

As might be expected, the question eventually arose: does specific clumping with dead animals of opposite type

induce nuclear reorganization or any other conjugation effects in living paramecia? The answer obtained to this question was quite strikingly positive (Metz, 1946b, 1947). While clumped to formalin-killed animals of one mating type, living animals of the opposite mating type unite to form pseudo-selfing pairs. The pseudo-selfing animals subsequently lose their ability to give the agglutinative mating reaction, they separate from the dead animals, and finally swim freely in the medium. These free swimming pairs can remain united for at least five hours. Union involves only the anterior or holdfast regions of the pair members (Figure 1a). Paroral cones form but these structures do not fuse. The two selfing pair members come from a single clone of a single mating type; furthermore both pair members give rise to clones of this same original mating type. Thus pseudo-selfing pair formation involves union of paramecia of the same mating type and is not regularly associated with permanent change of mating type.

Cytological examination shows that the pseudo-selfing pair members undergo meiosis and macronuclear breakdown (Metz, 1947) and that these nuclear changes are morphologically and temporally identical with corresponding changes in conjugating animals. Preliminary genetic studies (Jacobson, unpublished) indicate that the pseudo-selfers undergo autogamy.

Induced macronuclear breakdown and meiosis are not confined to the pseudo-selfers, for these changes can be induced independently of pseudo-selfing pair formation in single isolated living animals by formalin killed animals of opposite type. From this result it may be concluded with a reasonable degree of assurance that clumping with dead animals will induce autogamy directly and specifically in living animals of opposite mating type.

These effects of dead animals upon living animals are not causally related to the natural autogamy that occurs periodically in *P. aurelia*. In fact, they are not peculiar

to *P. aurelia*. Thus specific clumping between formalin-killed and living animals followed by meiosis, macronuclear breakdown and pseudo-selfing pair formation has been obtained in the Yale stocks of *Paramecium calkinsi* (Metz and Foley, unpublished). This study is of particular interest because spontaneous nuclear reorganization (endomixis, natural autogamy) has not been reported in *P. calkinsi* (Woodruff, 1921; Spencer, 1924) and could not be found in the stocks used in this study. Actually these changes have been induced in living Type II *P. calkinsi* only. Positive results have not been obtained in the reverse combination (dead type II plus living type I).

#### MECHANICS OF FERTILIZATION IN PARAMECIUM

It is now appropriate to attempt an interpretation of the facts outlined above. In conjugation, natural autogamy, pseudo-selfing and probably nuclear reorganization induced in single isolated animals, essentially the same series of events is observed, namely:

- (1) Loss of mating activity
- (2) Paroral cone formation
- (3) Meiosis
- (4) Macronuclear breakdown

The essentially identical nature of the series in the several types of behavior suggests a similar origin for these events in conjugation, natural autogamy, pseudo-selfing and reorganization induced in single animals. This similar origin is best visualized as a predetermined chain of reactions following from a common initiating mechanism. As stated elsewhere (Metz, 1947), the nature of the reaction between living and dead paramecia suggests that the common initiating mechanism may be an interaction of mating type substances. (It will be seen presently that natural autogamy is initiated through a separate mechanism). According to this view, interaction of mating type substances at the animal's surface would "acti-

vate" the *Paramecium* in the same sense that the spermatozoan activates the metazoan egg. In other words mating type substance interaction would perform a function in *Paramecium* analagous to that postulated by Lillie (1919) for the interaction of fertilizin and sperm receptor (Tyler's, 1942, sperm antifertilizin) in metazoan fertilization.

Support for this view was sought in a study of a non-conjugating race of *Paramecium aurelia* (Metz and Foley, 1947; unpublished). This study also suggests an interesting explanation of pseudo-selfing pair formation. The non-conjugating, "can't mate" or CM<sup>1</sup> animals give the initial mating or clumping reaction with normal animals. However, they do not form more permanent union with normal animals or undergo nuclear reorganization through association with normal animals. In other words, the CM animals can not be activated by animals of opposite mating type. The results of the investigation are summarized in Table I.

Table I Induction of pseudo-selfing and macronuclear breakdown in living normal and CM *P. aurelia* of one mating type by formalin-killed normal and CM animals of opposite type

Living	Normal	Normal	CM	CM
Dead	Normal	CM	Normal	CM
Initial clumping	+	+	+	+
Pseudo-selfing	+	+	—	—
Macronuclear breakdown	+	+	—	—

It was found that dead CM animals could induce pseudo-selfing in normal stocks (Table I). Indeed, even *living* CM animals induced pseudo-selfing pair formation in living normal animals. Thus the ability to induce pseudo-selfing is not peculiar to dead animals.

It was further found that the living CM animals never formed pseudo-selfing pairs when treated with dead normal animals (Table I). The mechanism previously sug-

<sup>1</sup> The writer is most grateful to Professor T. M. Sonneborn for supplying the CM stocks.

gested (Metz, 1947) to account for pseudo-selfing pair formation offers no ready explanation for this failure of the CM animals to self. Thus pseudo-selfing pair formation can not result directly from transfer of mating substances from dead to living animals unless the CM animals are endowed with very special properties.<sup>1</sup> It seems more reasonable, therefore, to attribute holdfast union, the union of pseudo-selfing, to interaction of separate substances which are not the mating type substances. It is not unreasonable to assume that these holdfast substances appear as a result of activation and are to be classed with loss of mating activity, paroral cone formation, meiosis and macronuclear breakdown.

As previously stated, dead normal (non-CM) animals activate single isolated normal animals. Likewise formalin-killed CM animals induce macronuclear breakdown and meiosis in single isolated normal animals. This demonstrates beyond question that the CM animals possess the activation initiating mechanism. However, the CM animals can not be activated by either living or dead normal animals of opposite mating type. Thus it appears that some block, *the CM block*, prevents activation from proceeding much beyond the initial step in CM animals. Depending upon the nature of the activation-initiating mechanism, at least two possibilities exist for the position of this CM block. If activation is initiated simultaneously in conjugants by interaction of a single system consisting of two complementary substances (Figure 2a) then the CM block must be placed in a position "internal" to the intact initiating system. This follows from the fact that CM animals can activate normal animals.

Conceivably activation of the two conjugants could involve interaction of two separate systems each consisting of two complementary substances, an "inducer" (I) and a "reactor" (R), such that each conjugant possessed the

<sup>1</sup> The possible nature of such special properties will be discussed in a later publication.

“inducer” of one system and the “reactor” of the other system. Interaction of I and R in one system would activate one conjugant, and interaction of R' and I' of

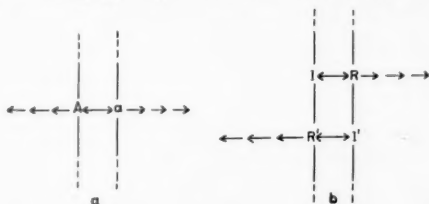


Fig. 2. Two possible activation-initiating mechanisms. Each series of arrows represents the main activation chain in one conjugant. (a) Simultaneous activation of conjugants by interaction of a single pair of surface substances. (b) Simultaneous activation of conjugants by interaction of two pairs of surface substances.

the other system would activate its mate (Figure 2b). In such a scheme the CM block could lie in the initiating mechanism rather than “internally,” if the CM animals lacked reactor (R).

The existence of the CM block, whatever its nature, is of considerable interest, and especially so since the CM animals regularly undergo natural autogamy. Because the CM animals can undergo natural autogamy, but can not be activated by animals of opposite mating type, it follows that different activation initiating reactions operate in sexually induced activation (conjugation, cytogamy and their experimental variants) and natural autogamy. Not only must different mechanisms initiate activation in conjugation and natural autogamy, but in natural autogamy the CM block is ineffectual. It appears, then, that in natural autogamy the main reaction chain is activated beyond the CM block, in other words between the CM block and the first bifurcation of the reaction chain. This will assume added interest if the CM block should prove to be independent of the activation initiating mechanism of conjugation. These relations are summarized in Figure 3.

In normal conjugation interaction of surface substances (position a, Figure 3) sets in motion a chain of reactions that ultimately branches into side reactions<sup>1</sup> (position d) leading to the several end effects of activation (position e), namely: holdfast substance formation (tentative),

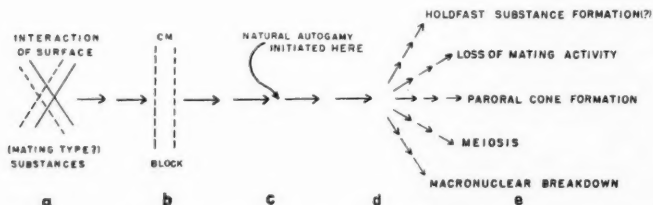


Figure 3. Scheme for activation in *Paramecium*. (a) Initiating reaction (mating type substance interaction?) in sexually induced activation. (b) CM block, here assumed to lie "internal" to the initiating reaction, (a). (c) Position where main chain is activated in natural autogamy. (d) Break-up of main activation chain into side reactions leading to (e) the various end effects of activation.

loss of mating activity, paroral cone formation, meiosis and macronuclear breakdown. In the CM animal the main chain is blocked at or near the initiating reaction by the CM block (position a or b, but assumed at b). Natural autogamy is not initiated through the same initial reaction but at some point in the main chain (possibly via a side reaction) beyond, or "internal" to the CM block (position c).

#### POSSIBLE RELATION OF MATING TYPE SUBSTANCES TO ACTIVATION

As previously suggested, interaction of mating type substances may initiate activation in *Paramecium*. The CM study supports this view. As shown above and also by Sonneborn (1942), holdfast union very probably does

<sup>1</sup> There is no evidence to indicate dependence of one side reaction upon another. However, it is possible, in fact, likely, that all side chains do not arise simultaneously and independently from the main chain. Indeed, the holdfast-substance side chain may arise between (b) and (c). This follows from the fact that holdfast union has never been observed in naturally autogamous animals. This may indicate that holdfast substances are not formed in natural autogamy or that holdfast substances are formed but that union requires the intimate contact found only in clumps or agglutinates.

not involve mating type substances. Since the CM animals can not be activated and can not form holdfast attachments, but can nevertheless activate single isolated normal animals, it follows that holdfast attachment or interaction of holdfast substances, is not essential for activation. The only other known substances that could be involved in activation, then, are the mating type substances (as defined by Metz, 1947). Since nothing is known of the number of these substances, this view is consistent with either of the activating mechanisms suggested in Figure 2. For the present it is either necessary to postulate that interaction of completely unknown and unsuspected substances initiate activation or to assume that mating type substance interaction initiates activation. The latter possibility is tentatively accepted in the scheme presented (Figure 3 position a).

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### THE AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES

An American Institute of Biological Sciences was formally established in February, 1948. The rapid advance of the biological sciences and their impact on human welfare have created new problems relating to the development and application of those sciences, according to a statement by the National Research Council. During recent years many biologists have recognized that the biological sciences suffer from the lack of a service organization, which would help the various biological societies to discharge more effectively those functions which are of common concern to them all, but which they cannot adequately exercise as individual societies. The new organization is designed to fill this need as well as to serve the biological sciences in other ways.

The Governing Board elected the following officers and Executive Committee:

Professor R. E. Cleland, Indiana University, *chairman*; Professor Elmer G. Butler, Princeton University, *vice-chairman*; Professor Wallace O. Fenn, University of Rochester; Drs. Theodore C. Byerly and F. P. Cullinan, United States Department of Agriculture. The two *ex officio* members are Dr. Detlev W. Bronk, professor of biophysics, University of Pennsylvania and Dr. J. S. Nicholas, professor of biology, Yale University.

Recognizing the potential importance of this new undertaking for the advancement of the biological sciences, and through them for all biologists, the National Research Council has not only endorsed the program, but has agreed to make available the general services of the Council. As a part of it the Institute will also provide biologists with an agency through which they can maintain close relations with governmental activities and with other fields of science represented within the Council.

